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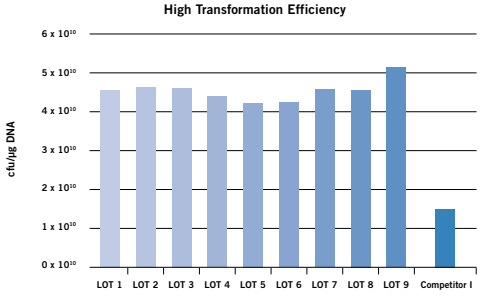

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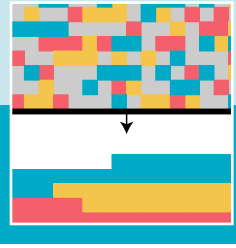
Science

Special Issue

Volume 308
6 May 2005
Number 5723

Distributed Computing

Computers processing data for the Oxford University project ClimatePrediction.net (see page 810). Scientific computing ventures in fields as varied as number theory, genomics, and particle physics have asked people to donate their computers' spare CPU cycles to create a virtual machine that dwarfs the top supercomputers. [Image: Chris Valentine/hockeyphotos.com; Martin Dzbor/KMi, Open University]



For related online content in STKE, see page 751 or go to www.sciencemag.org/sciext/computers/

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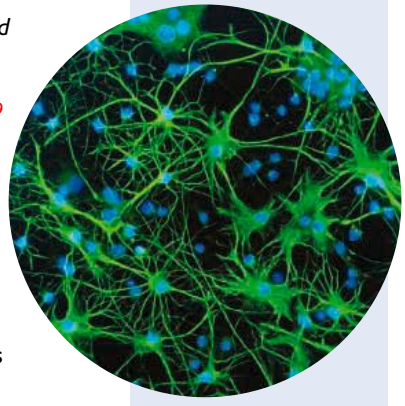

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Integrated Solutions — Automated Nucleic Acid Prep

A new generation of lab automation, as easy as A, B, C!

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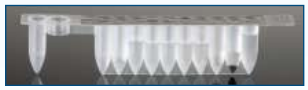

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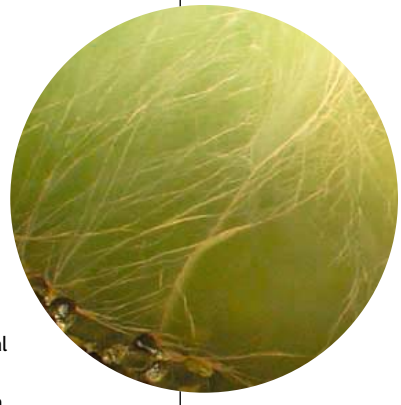


Science Express www.sciencexpress.org

Microbiology: Community Proteomics of a Natural Microbial Biofilm

R. J. Ram et al.

Analysis of 2033 proteins from the five predominant microbes in an acid mine drainage biofilm reveal many proteins involved in protein refolding and response to oxidative stress.

Medicine: Extension of Murine Life Span by Overexpression of Catalase Targeted to Mitochondria

S. E. Schriner et al.

In mice, expression of extra copies of an antioxidase enzyme in mitochondria reduces age-related decline and prolongs life span.

Medicine: A Mutation in the *TRPC6* Cation Channel Causes Familial Focal Segmental Glomerulosclerosis

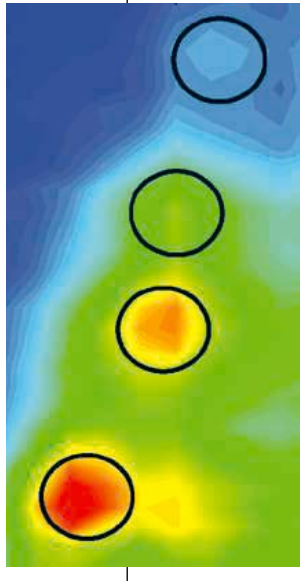
M. P. Winn et al.

An inherited form of a life-threatening kidney disorder is caused by a defect in a membrane protein thought to regulate calcium entry into cells.

Chemistry: The Rotational Spectrum and Structure of the HOOO Radical

K. Suma, Y. Sumiyoshi, Y. Endo

Spectrometry shows that the HOOO radical is Z-shaped, not a cis-structure as had been thought, providing a signature to look for this potentially important species in the atmosphere.



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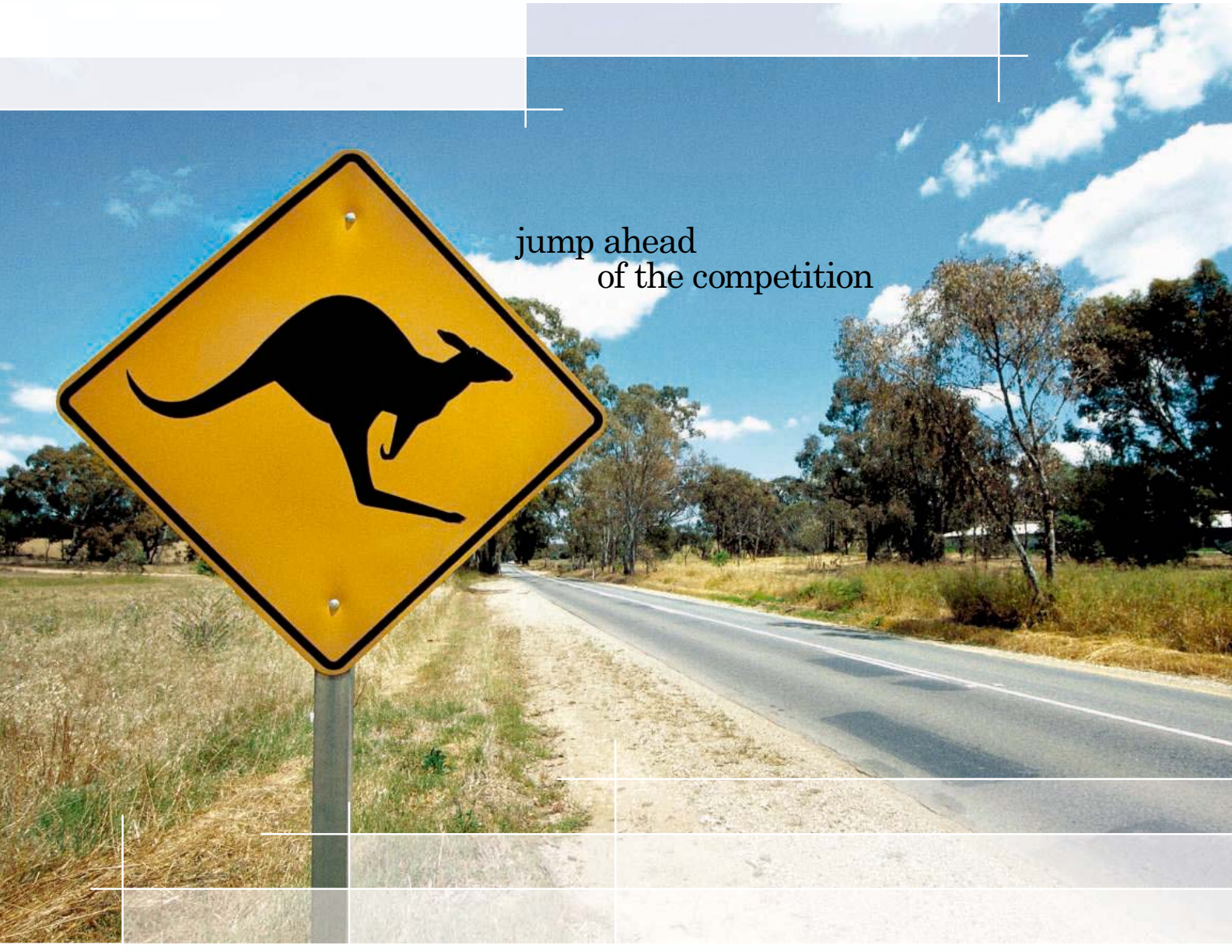

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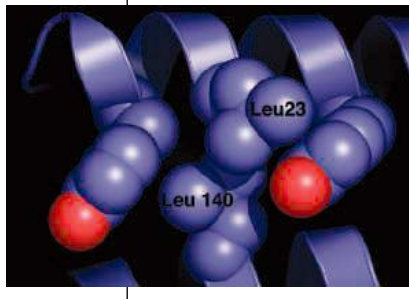

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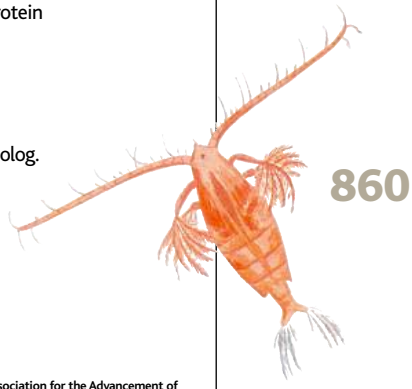

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Structures stir the ocean like a fine cocktail.

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With the development of new techniques, a whole new world is opening for future cave exploration.

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Researchers uncover genetic instructions for remaking worm body.

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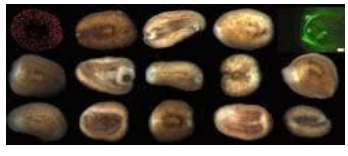
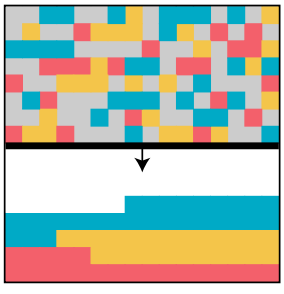
Science **119**, 623 (1954).



Regenerating genes.



Extracting information algorithmically.

science's stke www.stke.org Signal Transduction Knowledge Environment

Related Distributed Computing section page 809

Perspective: Text Mining for Metabolic Pathways, Signaling Cascades, and Protein Networks *R. Hoffmann, M. Krallinger, E. Andres, J. Tamames, C. Blaschke, A. Valencia*
Automatically extracting meaning is still a tricky process.

Perspective: A Life Science Semantic Web—Are We There Yet? *E. Neumann*
An enhanced "next generation" of the World Wide Web may better serve biologists for information management.

Connections Map Overview: *Drosophila* Wnt/Fz Pathways *R. DasGupta, M. Boutros, N. Perrimon*
New data lead to additions to this signaling pathway that is important in fly development. *related Perspective page 801; Research Article page 826*

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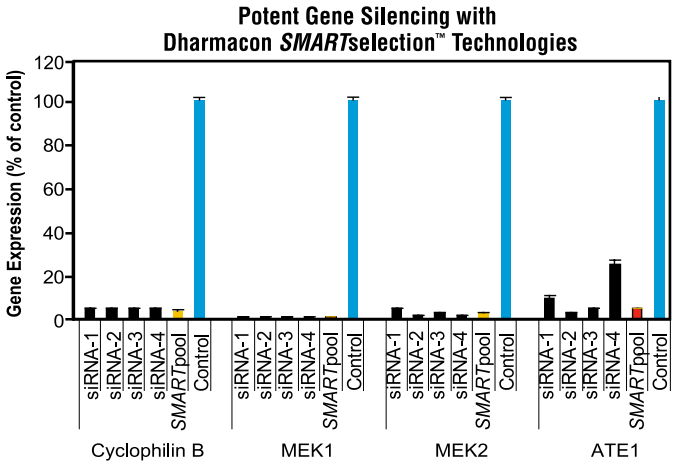

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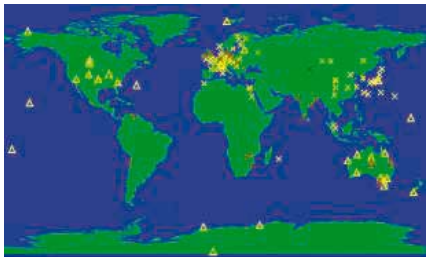
Excitons Prevail

When a material is confined to one dimension, its electronic band structure can exhibit features termed van Hove singularities, which have been invoked to explain the sharp absorption spectra in materials such as single-walled carbon nanotubes (SWNTs). This model predicts a sealike photoexcited state of free electrons and holes. Recently, however, support has emerged for an exciton picture, in which light absorption creates excited electrons that remain strongly correlated with the positive holes left behind. **Wang *et al.*** (p. 838) present firm evidence for the exciton model in isolated SWNTs. Their experiment takes advantage of the selection rules that exciton creation imposes on one- versus two-photon absorption. The two-photon spectra are consistent with exciton-binding strengths near 0.5 electron volt, which are much higher than in bulk semiconductors.

Implications of Sunny Days

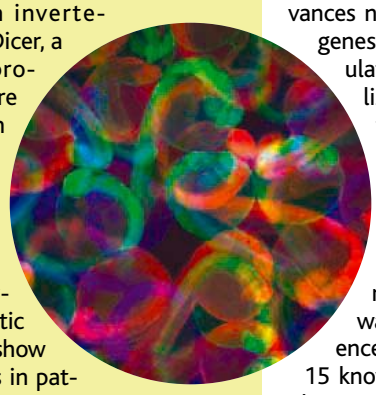
Many studies have reported direct or indirect evidence of a significant decrease in insolation (*S*), the amount of solar radiation reaching Earth's surface, during most of the past 40 years. How much *S* has varied, and why it may have changed, is poorly understood. **Pinker *et al.*** (p. 850; see the Perspective by **Charlson *et al.*** and the related Brevia by **Wielicki *et al.***) analyzed satellite records of *S* for the period from 1983 to 2001 and concluded that while there was a decrease in the earlier part of the record, the negative trend reversed around 1990 and was followed by an even larger increase. The recent upward trend is corroborated by **Wild *et al.*** (p. 847), who examined a large set of surface-based measurements of *S* starting in 1990. This dimming and subsequent brightening could have resulted from changes in cloud coverage, the abundance of atmospheric aerosols, or atmospheric transparency after explosive volcanic eruptions. Changes in insolation appear in numerous paleorecords from both high and low latitudes, but not all parts of the world responded concurrently. The differences in the nature and timing of their responses are thought to be important clues to the mechanisms that cause that asynchrony. **Wang *et al.*** (p. 854; see the news story by **Kerr**) present a precisely dated record of oxygen isotope variations in a stalagmite from Dongge Cave, China, which they interpret as a proxy for Asian Monsoon intensity. Their data, which extend back 9000 years to near the beginning of the Holocene, reveal important correlations between the strength of the monsoon and changes in solar output. They also discuss how the Dongge Cave record is related to climate records from Greenland, and implications for the mechanisms that have controlled the Asian Monsoon.



CREDITS (TOP TO BOTTOM): GIRALDEZ *ET AL.*, WILD *ET AL.*

Splicing Dicer

Small noncoding microRNAs (miRNAs) are potential regulators of gene function and have been shown to affect specific developmental processes in invertebrates. Null alleles of Dicer, a key enzyme in the production of miRNAs, are embryonic lethal in fish and mice. **Giraldez *et al.*** (p. 833, published online 17 March 2005) eliminated mature miRNAs in zebrafish by removing maternal and zygotic Dicer. These embryos show no overt abnormalities in patterning and cell fate specification but display severe defects in morphogenesis, particularly of the brain. Injection of a family of developmentally regulated miRNAs rescued brain morphogenesis.



A Reductionist Approach in Gene Screening

Cellular signaling pathways, such as Wnt in vertebrates or Wingless in flies, have traditionally been pieced together one step at a time. Technical advances now allow a more thorough probing of the genes whose products contribute to such a regulatory system. **DasGupta *et al.*** (p. 826, published online 7 April 2005; see the Perspective by **Fearon and Cadigan** and connection maps of the signaling pathways at *Science*'s STKE linked to the online paper) designed a high-throughput screen in *Drosophila* cells that evaluated effects on Wingless signaling when expression of nearly every gene (about 22,000 of them) was decreased, one by one, by RNA interference. The 238 genes identified included about 15 known components of the signaling pathway. The remaining group comprised approximately equal numbers of genes with known functions not previously associated with Wingless signaling. Half of the implicated genes appear to have orthologs in humans, and a substantial proportion of these human genes show mutations linked to disease.

Not Going with the Flow

Tiny zooplankton reside in the ocean at constant depth, despite the movement of currents. **Genin *et al.*** (p. 860) show that these organisms maintain their position by swimming against upwelling or downwelling currents at speeds of up to 10 body lengths per second. High-frequency, multibeam sonar was used to track more than 300,000 individual zooplankters. Combining these field measurements with a simulation model, the authors show that this behavior creates the dense zooplankton accumulations that become feeding grounds.

Maintaining Magma Temperatures

Earth's oldest rocks date to only about 4 billion years ago, but a few of these contain recycled zircons. These minerals formed in even earlier magmas, dating back to 4.4 billion years ago, or nearly the age of the Earth, and provide clues about Earth's earliest environment. **Watson and Harrison** (p. 841) have developed a means to probe the temperature of magmas from the titanium content of zircons and calibrated this thermometer

CONTINUED ON PAGE 755

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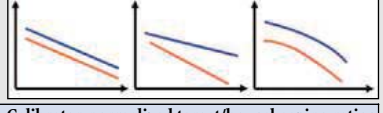
	Without efficiency correction	Efficiency correction with linear fit function	Efficiency correction with non-linear fit function
			
	Calibrator-normalized target/housekeeping ratios		
40 ng	1.03	1.18	1.41
8 ng	2.21	1.79	1.01
1.6 ng	6.00	4.17	1.17
Mean	3.08	2.38	1.21
S.D.	2.60	1.58	0.22
C.V.	**84.3%**	**66.4%**	**18.0%**

Figure 1: Impact of different PCR efficiency adjustments on accuracy of relative quantification. Total RNA was used for quantitative RT-PCR on the LightCycler® System. Sample data were evaluated with the LightCycler Relative Quantification Software, using the efficiency correction functions described above, to generate calibrator-normalized target/housekeeping ratios. The significantly lower Coefficient of Variation (C.V.) demonstrates the greater accuracy made possible by the LightCycler Software's use of efficiency corrections and a non-linear fit function.

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using both laboratory data and study of magmas with known or independently calibrated temperatures. They find that magmas that are more water-rich tend to be cooler. Application of this finding to these ancient zircons implies that they were formed from magmas that were similar in temperature and water content to those today. Thus, the nascent Earth may have been generating granitic magmas that were no hotter than those today.

Some Like It Hot

The efficiency of enzymes makes them attractive catalysts in industrial reactions. However, in many industrial applications the enzymes must operate at elevated temperatures, and designing active thermostable enzymes that maintain dynamic motions important for function is a challenge. **Korkegian *et al.*** (p. 857) have used a computational approach to identify three mutations that significantly stabilize the enzyme cytosine deaminase (CD) without reducing its catalytic efficiency. CD is a demanding model system because it forms an active dimer and displays complex folding behavior. Bacteria expressing the redesigned enzyme showed increased, temperature-dependent growth under conditions where an active enzyme would be required.

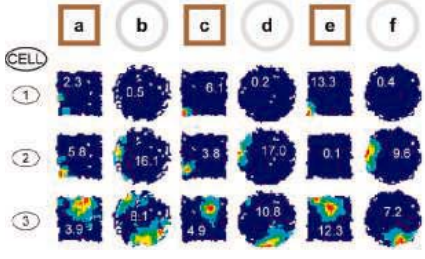
Reforming Fuel Cells

The hydrogen for fuel cells, at least in the near term, will come ultimately from the hydrogen available in hydrocarbon sources through a process called reforming. This process requires heat, so if this step can be completed "on board" a vehicle, it can take advantage of the heat provided by the fuel cell reaction to increase efficiency. However, the solid-oxide membrane fuel cells that can process hydrocarbons in this way have nickel anodes that tend to be deactivated by "coking," the depositing of unreacted carbon. **Zhan and Barnett** (p. 844, published online 31 March 2005) describe the preparation and operation of solid oxide fuel cells with a reformer layer (CeO_2/RuO_2) placed over the anode to produce CO and H_2 before the *iso*-octane fuel can reach the anode. They achieve power densities of 0.3 to 0.6 watt per square centimeter.

Swapping Partners for Perfect Pairing

Meiosis is the special "double" cell division in eukaryotes that results in the formation of haploid (germ) cells from diploid parent cells. Homologous chromosomes must pair during the first division so that they can be segregated equally between the two daughter cells. **Tsubouchi and Roeder** (p. 870) now show that, against expectations, initially nonhomologous pairs of chromosomes form during meiosis. Nonhomologous pairs are then resolved into homologous pairs as meiosis progresses, ensuring the correct segregation of chromosomes.

Spatial Memory Maps



Attractor networks have been the major hypothesis for the neural mechanism of memory. When rats explore two similar environments, neurons called place cells learn to distinguish between them (a process known as "remapping"). **Wills *et al.*** (p. 873; see the Perspective by **Poucet and Save**) provide evidence for coherent and complete transitions from one (attractor) state to another under conditions when sensory inputs change in a steady, incremental manner. Animals first explored two environments that differed in color, texture, and odor, as well as shape and, after the cells had remapped, were transferred to environments which varied along a single dimension (shape). The place cell representations of intermediate-shaped environments evolved into the (attractor) representations of either one or other initial shape: All simultaneously recorded cells coherently changed their firing pattern as a function of the intermediate shape. This direct evidence for the existence of attractor dynamics helps to provide a model for the representation of distinct contexts in context-dependent memory.

CREDIT: WILLS *ET AL.*

Turn to page 879

AVAILABILITY OF STANDARDIZED PREPARATIONS OF MESENCHYMAL STEM CELLS/MARROW STROMAL CELLS (MSCs)

The Center for Gene Therapy of Tulane University Health Science Center is pleased to offer research investigators standardized preparations of the adult stem cells from bone marrow stroma referred to as mesenchymal stem cells or marrow stromal cells (MSCs) under the auspices of a grant from the National Center for Research Resources of the N.I.H. The cells are provided for experimental purposes and not for administration to human subjects or for any commercial purposes.

Frozen vials of Passage 1 (P1) cells from one preparation of human MSCs are currently available. The cells are provided with (a) data from assays for infectious agents on blood of donors; (b) data developed in preparing the frozen vials of P1 cells; and (c) data obtained in expanding duplicate vials of the cells through two additional passages (to generate P2 and P3 cells). We will also provide our protocols for culturing the cells from frozen vials. The human MSCs are provided with a handling charge of $150 for 2 vials of about 1 million MSCs per vial. The Center can also provide (a) mouse MSCs (P5) from wildtype C57/ßl6 mice; (b) mouse MSCs (P5) from a transgenic C57 mouse ubiquitously expressing green fluorescent protein (GFP); (c) rat MSCs from Lewis rats (P5); (d) human MSCs (P3 or P4) transduced with a lentivirus to express GFP; and (e) human MSCs (P3 or P4) transduced with a lentivirus to express red fluorescent protein in mitochondria (MitoRed). The rodent MSCs are provided with a handling charge of $100 per vial of between 0.5 and 1 million cells. The handling charge can be waived on request, but the investigator must bear all shipping costs. Please address requests to Ms. Peggi Wolfe, Center for Gene Therapy, Tulane University Health Sciences Center, 1430 Tulane Avenue, SL-99, New Orleans, LA 70112. E-mail: wolfe@tulane.edu.

An Endless Frontier Postponed

Next month, U.S. scientists Vinton G. Cerf and Robert E. Kahn will receive computing's highest prize, the A. M. Turing Award, from the Association for Computing Machinery. Their Transmission Control Protocol (TCP), created in 1973, became the language of the Internet. Twenty years later, the Mosaic Web browser gave the Internet its public face. TCP and Mosaic illustrate the nature of computer science research, combining a quest for fundamental understanding with considerations of use. They also illustrate the essential role of government-sponsored university-based research in producing the ideas and people that drive innovation in information technology (IT).

Recent changes in the U.S. funding landscape have put this innovation pipeline at risk. The Defense Advanced Research Projects Agency (DARPA) funded TCP. The shock of the Soviet satellite Sputnik in 1957 led to the creation of the agency, which was charged with preventing future technological surprises. From its inception, DARPA funded long-term nonclassified IT research in academia, even during several wars, to leverage all the best minds. Much of this research was dual-use, with the results ultimately advancing military systems and spurring the IT industry.

U.S. IT research grew largely under DARPA and the National Science Foundation (NSF). NSF relied on peer review, whereas DARPA bet on vision and reputation, complementary approaches that served the nation well. Over the past 4 decades, the resulting research has laid the foundation for the modern microprocessor, the Internet, the graphical user interface, and single-user workstations. It has also launched new fields such as computational science. Virtually every aspect of IT that we rely on today bears the stamp of federally sponsored research. A 2003 National Academies study provided 19 examples where such work ultimately led to billion-dollar industries, an economic benefit that reaffirms science advisor Vannevar Bush's 1945 vision in *Science: The Endless Frontier*.

However, in the past 3 years, DARPA funding for IT research at universities has dropped by nearly half. Policy changes at the agency, including increased classification of research programs, increased restrictions on the participation of noncitizens, and "go/no-go" reviews applied to research at 12- to 18-month intervals, discourage participation by university researchers and signal a shift from pushing the leading edge to "bridging the gap" between fundamental research and deployable technologies. In essence, NSF is now relied on to support the long-term research needed to advance the IT field.

Other agencies have not stepped in. The Defense Science Board noted in a recent look at microchip research at the Department of Defense (DOD): "[DARPA's] withdrawal has created a vacuum . . . The problem, for DOD, the IT industry, and the nation as a whole, is that no effective leadership structure has been substituted." The Department of Homeland Security, according to a recent report from the President's Information Technology Advisory Committee, spends less than 2% of its Science and Technology budget on cybersecurity, and only a small fraction of that on research. NASA is downsizing computational science, and IT research budgets at the Department of Energy and the National Institutes of Health are slated for cuts in the president's fiscal year 2006 budget.

These changes, combined with the growth of the discipline, have placed a significant burden on NSF, which is now showing the strain. Last year, NSF supported 86% of federal obligations for fundamental research in IT at academic institutions. The funding rate for competitive awards in the IT sector fell to 16%, the lowest of any directorate. Such low success rates are harmful to the discipline and, ultimately, to the nation.*

At a time when global competitors are gaining the capacity and commitment to challenge U.S. high-tech leadership, this changed landscape threatens to derail the extraordinarily productive interplay of academia, government, and industry in IT. Given the importance of IT in enabling the new economy and in opening new areas of scientific discovery, we simply cannot afford to cede leadership. Where will the next generation of groundbreaking innovations in IT arise? Where will the Turing Awardees 30 years hence reside? Given current trends, the answers to both questions will likely be, "not in the United States."

Edward D. Lazowska and David A. Patterson

Edward D. Lazowska holds the Bill & Melinda Gates Chair in Computer Science & Engineering at the University of Washington. David A. Patterson holds the E. H. and M. E. Pardee Chair of Computer Science at the University of California, Berkeley, and is president of the Association for Computing Machinery. Both are members of the National Academy of Engineering and the President's Information Technology Advisory Committee, and past chairs of the Computing Research Association.

*The House Science Committee will consider these issues at a 12 May hearing on "The Future of Computer Science Research in the U.S." See http://www.cra.org/research.

10.1126/science.1113963

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EDITORS' CHOICE

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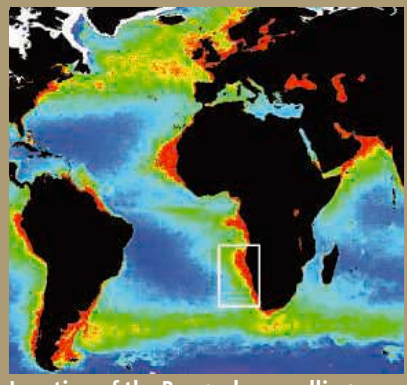
OCEAN SCIENCE

Reduced Nitrogen



Location of the Benguela upwelling (white box).

The growth of phytoplankton is limited by the loss of fixed nitrogen from the world's oceans. This loss occurs predominantly in zones of low oxygen (< 25 μM), such as the Black Sea, Chilean waters, and the Benguela upwelling off the Namibian coast. Classically, N_2 was thought to be produced by denitrification—the reduction of nitrate to N_2 by heterotrophic bacteria—but Kuypers *et al.* show that a large contribution may come via the anammox process: the anaerobic oxidation, carried out by bacteria known as *Planctomycetes*, of ammonium by nitrite. They present five corroborating strands of evidence. First, concentrations of nitrate drop at the bottom of the oxic zone; second, ammonium concentrations in the suboxic zone are low; third, water samples doped with [^{15}N]nitrate and [^{14}N]ammonium produced significant amounts of $^{14}N^{15}N$; fourth, ladderane lipids, characteristic of the anammoxosome membrane, were present; fifth, fluorescence in situ hybridization and ribosomal RNA sequence analysis revealed an abundance of *Planctomycetes* in the suboxic zone. One unknown is why there are anammox bacteria in the Benguela upwelling at depths where there is free oxygen (9 μM). Either these cells are quiescent, or there may be a suboxic microenvironment available, such as marine snow. — CA

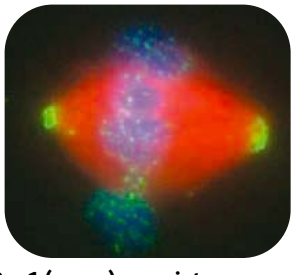
Proc. Natl. Acad. Sci. U.S.A. **102**, 6478 (2005).

CELL BIOLOGY

A Mitotic RNP

The mitotic spindle is an arrangement of cellular microtubules that acts as the physical scaffold used to partition chromosomes into the daughter cells during mitosis. Blower *et al.* find that an RNA-binding protein, Rae1, already known to be involved in the export of mRNA from the nucleus during interphase, also has a role in spindle assembly. Rae1 was isolated from *Xenopus* egg extracts as an activity required for spindle assembly. When it was depleted from egg extracts or from cells, mitotic spindle assembly was inhibited, and purified Rae1 stabilized microtubules in the presence of its nuclear import/export partners, the small GTPase Ran and importin β. Rae1 appears to be part of a large ribonucleoprotein (RNP) complex that controls microtubule dynamics; the association of RNA with the mitotic spindle is unanticipated but appears to be due to a structural requirement, perhaps as a second kind of scaffold. — SMH

Cell **121**, 223 (2005).



Rae1 (green) associates with spindle and aster microtubules (red).

APPLIED PHYSICS

Nanoparticle Films

Anti-reflective coatings are used in ophthalmic lenses, solar cells, optical data storage, and other applications in which reflections hamper device performance. Coatings are usually applied expensively via vacuum processes, and recently developed sol-gel methods still require multiple steps, including a thermal or chemical curing stage.

Krogman *et al.* have developed a simple process for applying anti-reflective films by spin-coating polymer substrates with metal oxide nanoparticles. Ceria or silica particles were added to water-based solutions of a penta-functional acrylate monomer to increase or decrease the refractive index, respectively, and were then deposited onto an acrylate substrate. In order to make thin, strong, and uniform films, the monomer solutions were doped with a second solvent to stabilize the colloidal particles and to enhance evaporation rates. By varying the concentration of nanoparticles, the authors tuned the refractive indices of the cured two-layer films and were also able to adjust the wavelength of minimum reflection. The nanoparticles hardened the films, too, making the coatings more resistant to wear. — MSL

Nanotechnology **16**, S338 (2005).

CHEMISTRY

Reactions That Float

Solvents are generally thought to accelerate bimolecular reactions by increasing the mixing of the substrates and by stabilizing key structural changes along the pathway. Both factors would seem to rely on intimate contact between the solvent and the reactants. For over a half-century, water has been known to accelerate some organic coupling reactions, such as Diels-Alder cyclization, but the effect has remained largely unexploited because of the poor aqueous solubility of most reagents.

Narayan *et al.* have achieved rate enhancements for a wide range of cycloadditions and ring-opening reactions simply by stirring the insoluble reaction partners in an aqueous suspension. Remarkably, several reactions involving azodicarboxylates are accelerated beyond the rate achieved by solvent-free mixing of miscible liquid reagents: Coupling of neat quadricyclane and dimethyl azodicarboxylate takes 2 days as compared to only 10 min "on water." Hydrogen bonding appears to increase the reaction rate, yet heterogeneity is a surprisingly important factor. When a suspension was homogenized by adding methanol, the reaction slowed down. A molecular explanation for the phenomenon is elusive, but the authors have encouraged those who make related observations to share their thoughts. — JSY

Angew. Chem. Int. Ed. 10.1002/anie.200462883 (2005).

MOLECULAR BIOLOGY

Silencing Mini-μ

Premature stop codons (PTCs) result in truncated proteins, species that would be extremely injurious.

CONTINUED ON PAGE 761

CREDITS: (TOP) KUYPERS *ET AL.*, *PROC. NATL. ACAD. SCI. U.S.A.* **102**, 6478 (2005); (BOTTOM) BLOWER *ET AL.*, *CELL* **121**, 223 (2005)

A post-transcriptional monitoring system—nonsense-mediated decay (NMD)—has evolved in eukaryotes to remove PTC-containing mRNAs before they can be translated. Immunoglobulin (Ig) genes are rearranged as part of normal lymphocyte development, and alleles containing PTCs are generated as nonfunctional byproducts of the process. Transcripts from these alleles are destroyed by NMD, but features of their extirpation suggest that something else is also suppressing these rogue mRNAs.

Bühler *et al.* have introduced PTCs into mouse Ig-μ minigenes and assayed their expression in tissue culture cells. They find that posttranscriptional NMD accounts for a 50% reduction in their expression. But they also find that 50% of the suppression occurs at the level of transcription and is mirrored by chromatin features associated with gene silencing: the loss of histone acetylation and an increase in methylation of histone H3 on the lysine-9 residue in the vicinity of the PTC-containing mini-genes. Repression of putative small interfering RNAs (siRNAs) by overexpression of the siRNase 3'hExo abrogates the PTC-suppression effect, suggesting that RNA interference–related mechanisms may be involved. — GR

Mol. Cell **18**, 307 (2005).

PSYCHOLOGY

Happiness in the Civil Service

It is not surprising that negative emotional states, such as stress or depression, are associated with a higher risk of unhealthy conditions, such as cardiovascular disease. We can assess stress (cortisol) and depression (psychiatric diagnosis) in objective ways, but how can we ascertain whether positive affect (happiness) is healthful? In beginning to address this question, Steptoe *et al.* have collected two data sets from over 200 British civil servants (mostly happy and healthy). One contains aggregate measurements (35 time points in a working day) of physiological (cortisol) and psychological (self-ratings) status, and the other contains similar measurements recorded in a laboratory mental stress test (modified Stroop task). [See also the Day Reconstruction Method of Kahneman *et al.*, Reports, 3 December 2004, p. 1776.] They find that cortisol and plasma fibrinogen (a predictor of coronary heart disease) levels were inversely related to happiness and that these correlations were independent of psychological distress, supporting the idea that positive affect may be associated with neuroendocrine and cardiovascular indicators of well-being. — GJC

Proc. Natl. Acad. Sci. U.S.A. **102**, 6508 (2005).

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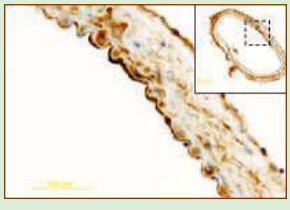
Vascular Effects of Stress

Atherosclerotic plaques, which develop in response to a localized inflammatory response, occur at regions of disturbed blood flow. Fluid shear stress stimulates the binding of endothelial cell integrins to the subendothelial extracellular matrix (ECM), leading to activation of the nuclear factor-κB (NF-κB) signaling pathway and transcription of target genes. Noting that endothelial cells express multiple integrins that bind to matrix proteins and that inflammation promotes the deposition of fibronectin and fibrinogen into the subendothelial ECM, Orr *et al.* found that changes in subendothelial matrix composition and activation of NF-κB target genes occurred at regions of disturbed flow in vivo before other atherosclerotic changes and were most pronounced in atherosclerosis-prone mice fed a high-fat diet. Fluid shear stress promoted phosphorylation and translocation to the nucleus of NF-κB in bovine aortic endothelial cells cultured on fibrinogen or fibronectin. In contrast, shear stress, acting through integrin $\alpha 2\beta 1$, promoted activation of the p38 protein kinase in cells grown on collagen, leading to reduced NF-κB activation. Intriguingly, NF-κB activation in cells grown on fibronectin could be blocked by treatment with a peptide that alters matrix structure and stimulates p38, suggesting that modification of the ECM with external factors (and localized activation of p38 at integrin adhesion sites) could provide a novel approach to treating atherosclerosis. — EMA



Arterial deposition of fibrinogen (brown) in a mouse prone to atherosclerosis on a high-fat diet.

J. Cell Biol. **169**, 191 (2005).

CREDITS: ORR *ET AL.*, *J. CELL BIOL.* **169**, 191 (2005)

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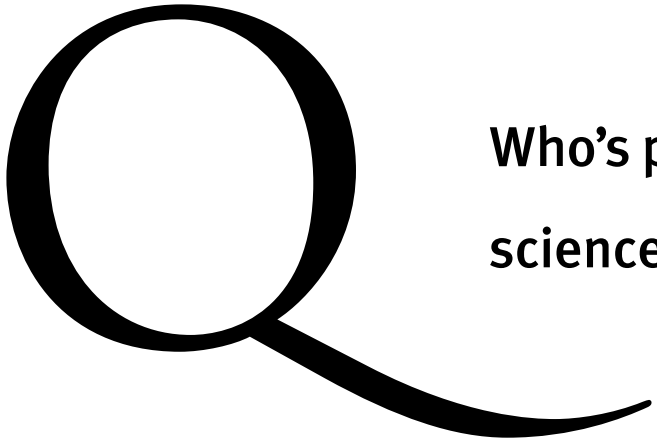

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>
> Brendan Curran, physics teacher and AAAS member

AAAS

Photo: Jane Jang & Nick Mastorakis

Brendan Curran, with his physics class at Herricks High School, New Hyde Park, New York

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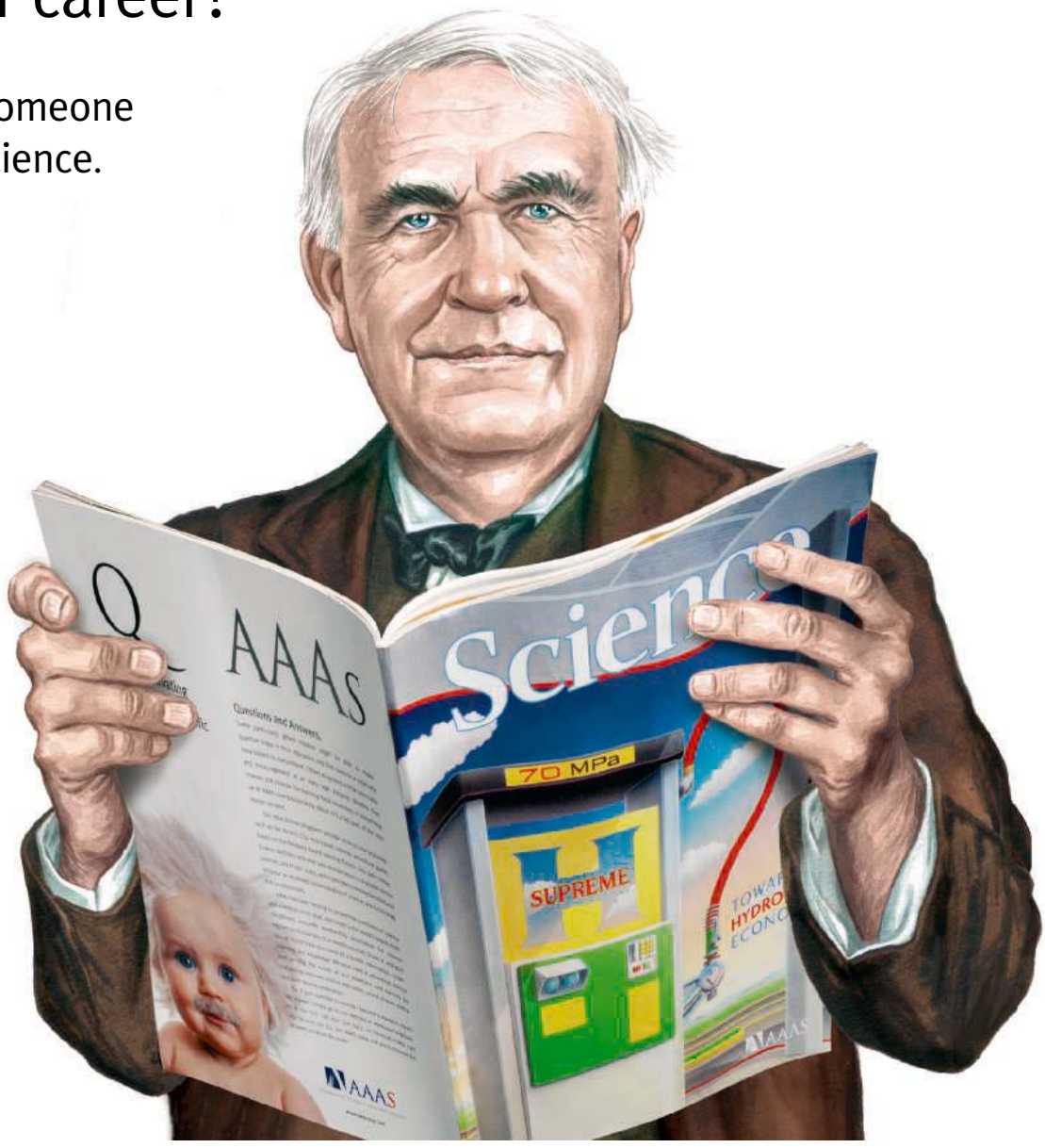

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NetWatch

edited by Mitch Leslie

WEB ARCHIVE

Bird Journals Roost Online

Researchers who want to browse the historic bird literature should take a gander at the Searchable Ornithological Research Archive (SORA), hosted by the University of New Mexico, Albuquerque. This Web library, named for the marsh-dwelling sora (above), holds more than 100 years' worth of *The Condor*, *The Auk*, and *The Wilson Bulletin*, along with shorter spans of the *North American Bird Bander*, *Studies in Avian Biology*, and other ornithological titles. A search function lets you scan the full texts of all the journals, and you can download articles as PDFs or in the more concise DjVu format, which requires a free plug-in to view. The newest volumes date to 2000.

elibrary.unm.edu/sora/index.php

RESOURCES

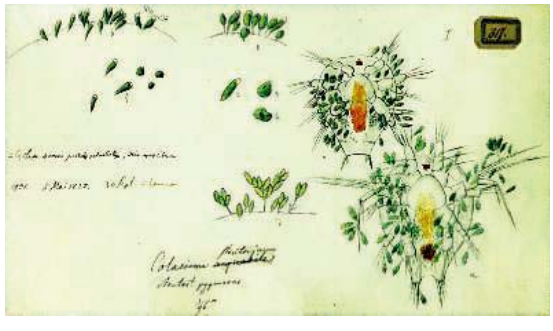
Bad Stats, Bad Medicine

The recent ruckus over the safety of the pain relievers Vioxx and Celebrex makes the opinionated Web site Improving Medical Statistics a timely read. Eric Roehm, a cardiologist from Round Rock, Texas, exposes statistical gaffes, shoddy study designs, and unwarranted conclusions that slipped past peer review and into the pages of top journals. For example, the doctor's warning that pregnant women should abstain from alcohol stems from a flawed 1984 study that didn't factor out the effects of smoking. Even the 2001 paper that first raised questions about the safety of Vioxx and Celebrex has a weakness: The researchers compared the treatment group from one study to placebo groups from other trials.

www.improvingmedicalstatistics.com/index.html

RESOURCES

Portrait of a Protist



"Photosynthetic" and "hyperactive" don't usually go together, but they're apt adjectives for the microscopic *Euglena* and its relatives, which carry chloroplasts but can chase down their fellow pond dwellers. The peripatetic protists are the subject of the Euglenoid Project Web site. A primer introduces peculiarities of euglena behavior and anatomy. Visitors can also check out the original euglena sketches (right) by German biologist Christian Ehrenberg—who named the creatures in 1830—or screen movies of cells on the move or snarfing other protists. With interactive keys and synopses of most genera, the site swarms with information for taxonomists. It will soon expand to include full-text versions of most classic euglena literature, says co-creator Richard Triemer of Michigan State University in East Lansing.

www.plantbiology.msu.edu/triemer/Euglena/Index.htm

LINKS

Molecular Biologist's Companion

The Web abounds with an ever-growing number of molecular biology and medical databases. For help finding the one you need, try this annotated directory of links compiled by Josef Koenig of the Medical University in Vienna. Under categories such as genomics, pharmacology, and ethics, the directory lists annotated links to hundreds of sites. To find out how bacteria handle toxins, for example, hop over to the database hosted by the University of Minnesota that records bacterial breakdown pathways for nearly 900 compounds. Some entries include links to publications on the database.

www.meddb.info

EXHIBITS

A Frigid Banner Year

Neither marauding wolves, nor temperatures as low as –46 degrees Celsius, nor overdue supply ships stayed the explorers at Fort Conger in northwestern Greenland (right) from their meteorological rounds. In 1882 and 1883, U.S. personnel at this isolated station and researchers at other sites across the Arctic recorded air temperature, barometric pressure, wind speed, and other variables as part of the first International Polar Year. At this site from the National Oceanic and Atmospheric Administration, history buffs can learn more about this pioneering project, and researchers can download the original data.

The project's goal was to share environmental measurements from different locales, and 11 countries teamed up to staff Arctic observing stations. Their readings provide a snapshot of the far north before human-induced global warming began. Besides data, the site holds an archive with more than 200 photos, maps, and drawings that provide a glimpse of life at the stations. Paintings even record the deaths of three members of the Fort Conger expedition; only seven of the 25 members of the party were alive when rescuers arrived.

www.arctic.noaa.gov/aro/ipy-1

Send site suggestions to netwatch@aaas.org. Archive: www.sciencemag.org/netwatch

CREDITS (TOP TO BOTTOM): DANA GARDNER; NOAA; DAVID LAZARUS/MUSEUM FÜR NATURKUNDE/HUMBOLDT UNIVERSITY

This Week

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Computing grid invites problems

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Celera releases its genome data

PUBLIC HEALTH

A Heavyweight Battle Over CDC's Obesity Forecasts

How many people does obesity kill?

That question has turned into a headache for the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia: In the past year, its scientists have published dueling papers with conflicting estimates on obesity-associated deaths—the first three times greater than the second. The disagreement, some fear, is undermining the agency's health warnings.

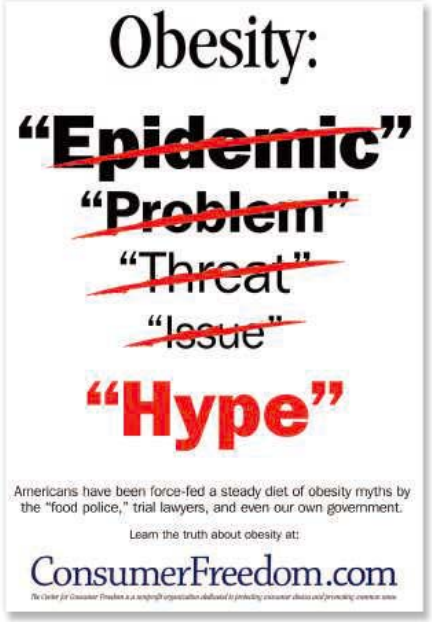
The bidding on obesity's annual death toll started at a staggering 400,000—the number cited in a CDC paper co-authored by CDC chief Julie Gerberding in 2004. But dissent prompted an internal inquiry, and CDC decided this year to lower the number to 365,000. That was still too high for some CDC analysts, who together with colleagues at the National Cancer Institute (NCI) in Bethesda, Maryland, published a new figure on 20 April—112,000 deaths. The low estimate is spawning other problems, though. A food-industry interest group is touting it as evidence that obesity is not so risky. Even researchers who favor the low number worry that it will lead to complacency.

After trumpeting the highest estimate a year ago and warning that obesity deaths were poised to overtake those caused by tobacco, CDC officials now say that numbers are unimportant. The real message should be that "obesity can be deadly," says George Mensah, acting director of CDC's National Center for Chronic Disease Prevention and Health Promotion. "We really add to the confusion by sticking to one number."

But some of CDC's own scientists disagree. "It's hard to argue that death is not an important public health statistic," says David Williamson, an epidemiologist in CDC's diabetes division and an author on the paper with the 112,000 deaths estimate.

Calculating whether obesity leads directly to an individual's demise is a messy proposition. To do so, researchers normally determine by how much obesity increases the death rate and what proportion of the population is obese. Then they apply that to the number of deaths in a given time, revealing excess deaths due to obesity. Both studies use that approach, but methodological differences produced big disparities between the two papers—one by epidemiologist Ali Mokdad, Gerberding, and their CDC colleagues, published in the *Journal of the American Medical Association* (*JAMA*) on 10 March 2004, and the new estimate by CDC epidemiologist Katherine Flegal and colleagues at CDC and NCI, published in *JAMA* on 20 April.

Both relied on data about individuals' weight and other measures from the National Health and Nutrition Examination Survey (NHANES), which has monitored the U.S. population since the 1970s. The Mokdad group used the oldest, NHANES I. Flegal's group also used two more recent NHANES data sets from the 1980s and 1990s. Her method found fewer obesity-associated deaths—suggesting that although obesity is rising, some factor, such as improved health care, is reducing deaths.



Feeding on confusion. An ad campaign by a food industry–supported group seeks to exploit discrepancies in estimated obesity deaths.

Other variations in methodology proved crucial. For example, the two groups differed in their choice of what constitutes normal weight, which forms the baseline for comparisons. Flegal's team adopted the definition favored by the National Institutes of Health and the World Health Organization, a body mass index (BMI) between 18.5 and less than 25. The Mokdad team chose a BMI of 23 to less than 25; this changed the baseline risk of death, and with it, deaths linked to obesity.

In their paper, the Mokdad authors said they selected that narrower, heavier range because they were trying to update a landmark 1999 *JAMA* paper on obesity led by biostatistician David Allison of the University of Alabama, Birmingham, and chose to follow Allison's methodology. (CDC spokesperson John Mader said that Mokdad and his co-authors were not available to be interviewed.) "There's no right answer" to which BMI range should be the "normal" category, says Allison. He felt his choice was more "realistic," and that expecting Americans to strive for even lower BMIs might be asking too much. But that relatively small difference in BMI had a big effect on the estimates: Had Flegal's team gone with the 23-to-25 range, she reported, the 112,000 deaths estimate would have jumped to 165,000.

The scientists also diverged sharply in how they tackled age. It's known that older individuals are less at risk and may even benefit from being heavier: A cushion of fat can keep weight from falling too low during illness. And young obese people tend to develop more severe health problems, says David Ludwig, director of the obesity program at Children's Hospital in Boston.

Flegal's group took all this into account by assigning risks from obesity to different age groups. Stratifying by age meant that when Flegal turned to actual death data—all deaths from the year 2000—she was less likely to count deaths in older age groups as obesity-related.

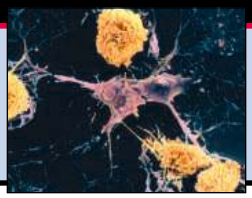
Allison concedes that in retrospect, his decision not to stratify by age was a mistake. And it had a big impact on the estimates. "Very minor differences in assumption lead to huge differences in the number of obesity-induced deaths," says S. Jay Olshansky, a biodemographer at the University of Illinois, Chicago.

Olshansky, Allison, and Ludwig published their own provocative obesity paper in *The New England Journal of Medicine* in March. It argued that U.S. life expectancy could begin decreasing as today's obese children grow up and develop obesity-induced diseases, such as diabetes and heart disease (*Science*, 18 March, p. 1716).

But Olshansky now says that in light of Flegal's recent paper on obesity deaths and a companion paper that she, Williamson, and other CDC scientists authored in the same issue of *JAMA*, his life expectancy ▶

CREDIT: THE CENTER FOR CONSUMER FREEDOM

778
When the brain's support staff go awry



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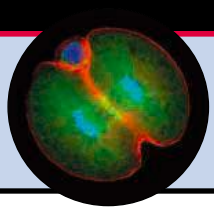
When do embryonic cells know their fate?



787
Ancient monsoons in a stalagmite

forecasts might be inaccurate.

The companion paper, led by CDC's Edward Gregg, examined how much cardiovascular disease was being driven by obesity. The findings were drawn from five surveys, most of them NHANES, beginning in 1960 and ending in 2000, and they dovetailed with the conclusions in Flegal's 112,000 deaths paper. All heart disease risk factors except diabetes were less likely to show up in heavy individuals in recent surveys than in older ones. That suggests, says Allison, that "we've developed all these great ways to treat heart disease" such as by controlling cholesterol. This could also explain, he and others say, why NHANES I led to much higher estimates of obesity-associated deaths than did NHANES I, II, and III combined. Although obesity rates are rising, obesity-associated deaths are dropping.

Ludwig disagrees that this trend will necessarily continue or that Gregg's paper disproves the one he co-authored with Olshansky. Type 2 diabetes, which is becoming more common in youngsters, "starts the clock ticking towards life-threatening complications," he notes.

Olshansky is uncomfortable with the kind of attention Flegal's 112,000 estimate is getting. "It's being portrayed," he says, as if "it's OK to be obese because we can treat it better." In fact, one of Flegal's conclusions that sparked much interest—that being overweight, with a BMI of 25 to 30, slightly reduced mortality risk—had been suggested in the past.

Heavy duty. Being obese in childhood increases the likelihood of health problems such as diabetes later on.

Certainly, food-industry groups are thrilled by Flegal's work. "The singular focus on weight has been misguided," says Dan Mindus, a senior analyst with the Center for Consumer Freedom, a Washington, D.C.–based nonprofit supported by food companies and restaurants. Since Flegal's paper appeared, the center has spent $600,000 on newspaper and other ads declaring obesity to be "hype"; it plans to blanket the Washington, D.C., subway system with its ad campaign.

Some say that CDC needs to choose one number of deaths and stand behind it. "You don't just put random numbers into the literature," says antitobacco activist and heart disease expert Stanton Glantz of the University of California, San Francisco, who disputed the Mokdad findings.

Scientists agree that Flegal's study is superior, but it may also be distracting, suggests Beverly Rockhill, an epidemiologist at the University of North Carolina, Chapel Hill. Even if obese individuals' risk of death has been overplayed in the past, she says, we ought to ask: "Are they living a sicker life?"

–Jennifer Couzin

ASTRONOMY

Picture-Perfect Planet on Course for the History Books

Look closely at the faint red speck of light in this false-color photo. It's the first image ever of an exoplanet—a planet outside our own solar system. The 8-million-year-old world, about the size of Jupiter but five times as massive, has water vapor in its atmosphere and circles its mother brown dwarf star every 2500 years or so at a distance of 8 billion kilometers. The whole system is 230 light-years away in the constellation Hydra. The planet's name? 2M1207b, but that may change.

A European-American team of astronomers led by Gaël Chauvin of the European Southern Observatory took the infrared photo in April 2004 using ESO's 8.2-meter Very Large Telescope (VLT) in Chile, outfitted with a revolutionary system to compensate for atmospheric turbulence. Until now the team couldn't rule out the possibility that the red dot was a background object, unrelated to the brown dwarf. But new VLT measurements confirm that the two objects are moving through space together, and independent Hubble Space Telescope data released on 2 May at an exoplanet workshop in Baltimore, Maryland, all but clinch the case. "At the 99.9% level, I agree this is probably the first image of an extrasolar planet," says Eric Becklin of the University of California, Los Angeles (UCLA), who was not involved in either study.

First light. Infrared image shows portrait of an extrasolar planet *(left)*.

But is it really a planet and not, say, another brown dwarf star? According to theoretical models for inferring the mass of young, low-mass objects from their infrared spectra, 2M1207b is only five times as massive as Jupiter. That's well below the 13.6-Jupiter-mass cutoff the International Astronomical Union uses to distinguish planets from brown dwarfs. "The possibility that this object is a brown dwarf is out of the box," says Glenn Schneider of the University of Arizona in Tucson, who presented the Hubble results. If anything, "the models may well overestimate the masses at very low mass," says Gibor Basri of the University of California, Berkeley. Together with his student Subu Mohanty and others, Basri developed a new way of determining masses of substellar objects by deducing their surface gravity from detailed spectroscopic measurements. Their results indicate that bodies like 2M1207b are probably even less hefty than current theoretical models suggest.

With its claim to fame assured, says co-discoverer Benjamin Zuckerman of UCLA, the team hopes to give the planet a name better suited to its historic status. "Anyone with a bright idea is welcome to suggest it," he says.

–Govert Schilling

Govert Schilling is a writer in Amersfoort, the Netherlands.

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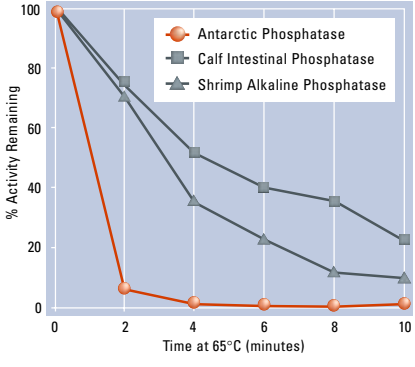

10 units of each phosphatase were incubated under recommended reaction conditions (including DNA) for 30 minutes and then heated at 65°C. Remaining phosphatase activity was measured by p-nitrophenyl-phosphate (pNPP) assay.

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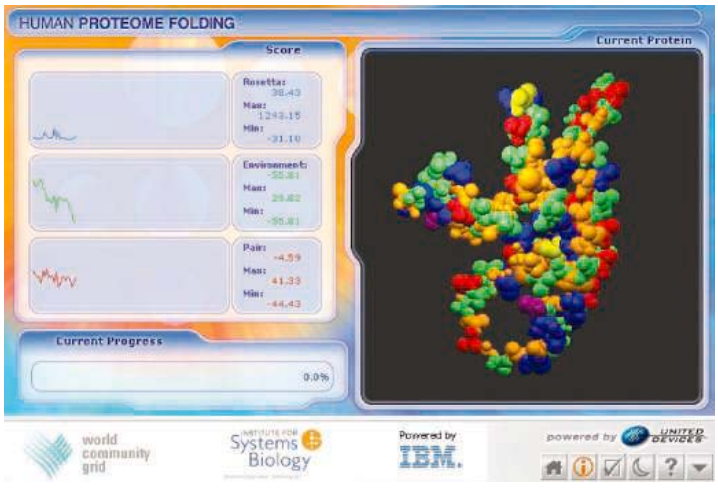
COMPUTING

IBM Offers Free Number Crunching For Humanitarian Research Projects

CAMBRIDGE, U.K.—When researchers have a project that involves a lot of number crunching, they usually have to think small. They compress data and algorithms to make the best use of expensive computer time. Now the computer giant IBM is offering researchers who meet certain criteria a chance to do the opposite: to think big—supercomputer big—and it will provide access to the computing power for free.

The company's philanthropic arm has launched an effort known as World Community Grid (WCG) to support research projects with humanitarian goals. "We aim to take the most cutting-edge technologies and use them in the public interest," says Stanley Litow, president of the IBM International Foundation. The computing power comes courtesy of many thousands of ordinary computer users around the world who freely donate their computers to a project at times when they would otherwise sit idle. Linked by the Internet, the grid gains power as it accumulates machines. Last month WCG signed up its 100,000th computer.



Group effort. Small computers are being linked in huge networks to analyze protein folding and other puzzles.

WCG uses the same technique as projects such as SETI@home and Climate-Prediction.net, which install a screen saver on computers to sift radio signals for extraterrestrial messages or model climate change (see p. 810). The difference is that WCG has a permanent infrastructure and can run five or six projects at once. IBM created the open grid because "we found that a lot of projects were dying on the vine in the absence of computing power," says Litow.

WCG is not the first grid freely available to researchers. The company United Devices in Austin, Texas, which creates similar links for the pharmaceutical, oil, and financial industries, set up Grid.org in 2001 and has since signed up more than 3 million machines. Grid.org's first project was to scan 3.5 billion molecules for potential as drugs against cancer. Chemist Graham Richards of Oxford University in the U.K., who led the effort, says participants "employed more computing power than the whole world pharmaceutical industry" can bring to bear on such problems. Richards says the project found lots of promising molecules and is now embarking on the more painstaking process of synthesizing the molecules and testing them in vitro.

The Oxford team also used Grid.org to search for drugs against anthrax and, in collaboration with IBM, smallpox—a project that screened 35 million potential drug molecules to find 44 strong candidates in a matter of weeks. "The smallpox experiment was such a success," says Viktors Berstis, IBM's technical head of WCG, that IBM decided to set up its own grid. WCG was launched in November 2004, with help from United Devices, and its first task was the Human Proteome Folding Project. Devised by researchers at the Institute for Systems Biology in Seattle, Washington, the folding project predicts structures for the thousands of protein sequences uncovered by the Human Genome Project. At a symposium in Seattle last week, the institute announced that the project had already calculated 50,000 structures. Its goal—100,000 to 150,000 structures—would take 100,000 years to complete if the institute relied on its own computing power.

Interested researchers can propose projects at www.worldcommunitygrid.org, and IBM has assembled a high-powered advisory board, including David Baltimore, president of the California Institute of Technology in Pasadena, and Ligia Elizondo, deputy director of the United Nations Development Programme, to sift through the proposals. The board is meeting this week and hopes to have a first slate of new projects in a few months. Berstis says he hopes eventually to sign up as many as 10 million computers. "Most researchers haven't even thought of this kind of massive computing power," he says. It's time to think big.

–DANIEL CLERY

CREDIT: WORLD COMMUNITY GRID

ScienceScope

Democrats Protest Limits on WHO Advisory Panels

Some Democrats in Congress want the Bush Administration to halt what they see as efforts to exert political control over science.

Their focus is a 1-year-old policy on sending federal scientists to meetings of the World Health Organization (WHO). In the past, WHO would directly invite individuals from the Department of Health and Human Services (HHS) to serve as advisers on topics such as avian flu and potentially cancer-causing chemicals. But in April 2004, then–HHS secretary Tommy Thompson's global health chief, William Steiger, announced that invitations needed to go to his office, which would choose the appropriate experts. The policy upset researchers at the National Institutes of Health and the Centers for Disease Control and Prevention as well as outside public health leaders and scientific groups.

In a 28 April letter to new HHS Secretary Michael Leavitt, the 11 Democrats on the House Science Committee ask him to rescind the policy or explain the value of what legislators call a "counterproductive" and "potentially dangerous" policy. An HHS spokesperson said the department expects to respond "in an appropriate time frame."

–JOCELYN KAISER

NIH Wants Your Papers Now

The National Institutes of Health's (NIH's) new push to expand public access to papers it funds kicks in this week. As of 2 May, NIH-funded investigators are requested to submit copies of final, accepted journal manuscripts to NIH (www.nihms.nih.gov), which will post them in NIH's PubMed Central papers archive no more than 12 months after they're published in the journal.

NIH announced the policy in February after a 6-month battle between open-access advocates and journal publishers, who say the policy violates copyrights and will put them out of business. One question is how authors will interpret NIH's recommendation that they ask NIH to post their papers "as soon as possible," regardless of when the journal allows free online access to the full text. Also unknown is how well the National Library of Medicine will cope with the flood of manuscripts, expected to number at least 60,000 a year.

–JOCELYN KAISER

SCIENCE RESOURCES

Chemists Want NIH to Curtail Database

The American Chemical Society (ACS) wants the U.S. government to shut down a free database that it says duplicates the society's fee-based Chemical Abstracts Service (CAS). Government officials defend the site, called PubChem, saying the two serve different purposes and will complement, rather than compete with, each other. But ACS officials are hoping to convince Congress to stop PubChem unless the government scales it back.

PubChem was launched last fall by the National Institutes of Health (NIH) in Bethesda, Maryland, as a free storehouse of data on small organic molecules. It is a component of the Molecular Libraries Initiative, which is a part of NIH Director Elias Zerhouni's road map for translating biomedical research. So far, PubChem includes information on 650,000 compounds, such as structures and biological assays, as well as links to PubMed, NIH's free biomedical abstracts database. It will grow to include data from the Molecular Libraries centers, which aim to screen thousands of molecules for biological activity. NIH expects basic researchers to use PubChem to identify chemicals they can use to explore how genes and cells work.

Boiling point. ACS's Madeleine Jacobs says NIH's PubChem goes too far.

But ACS claims PubChem goes far beyond a chemical probes database. It is, ACS says, a smaller version of CAS, which employs more than 1200 people in Columbus, Ohio, and makes a significant contribution to the society's $317 million in annual revenue from publications. Institutional subscribers receive data on 25 million chemicals, including summaries written by CAS experts and links to chemistry journal abstracts. Like CAS, PubChem assigns each chemical a unique identifying number, and until a few weeks ago, the sites even looked quite similar, says ACS Chief Executive Officer Madeleine Jacobs. Claiming that PubChem could wipe out CAS, Jacobs argues that NIH should abide by its stated mission of storing only data from the Molecular Libraries Initiative and other NIH-funded research.

NIH officials counter that PubChem indexes a set of biomedical journals that overlaps only slightly with those CAS indexes and, unlike CAS, does not provide curated information on patents or reactions. "They have a vast amount of information that PubChem would never dream of including," says Francis Collins, director of the National Human Genome Research Institute. PubChem's focus on biological information such as protein structures and toxicology is complementary, he says. NIH has offered to link entries in PubChem to CAS, but ACS says that wouldn't help.

ACS has enlisted Ohio's governor, Republican Bob Taft, as well as the state's congressional delegation to push its case. The legislators sent a letter on 8 March to Health and Human Services Secretary Michael Leavitt arguing that PubChem could pose "direct and unfair competition" with CAS. The lawmakers compare it to PubScience, a Department of Energy abstracts database that was shut down in 2002 after House appropriators decided it violated rules prohibiting the government from duplicating private services. ACS was part of that lobbying campaign.

NIH officials are worried that PubChem could suffer the same fate and hope to make their case this month to Senator Mike Dewine (R–OH). Jacobs, for her part, wants NIH to "stick to its mission" and cut back the scope of PubChem. If not, she promises "to bring to bear all of our influence and resources."

–JOCELYN KAISER

EUROPEAN POLICY

Panel Gives Thumbs-Down to European Institute of Technology

BERLIN—Efforts to create a European Institute of Technology (EIT) to compete with the Massachusetts Institute of Technology (MIT) could do more harm than good to science in Europe, an advisory panel told the European Commission last week. The idea for a so-called EIT was proposed in February as part of the relaunch of the so-called Lisbon strategy, designed to boost Europe's flagging economy. The strategy highlights research as a catalyst for economic growth, and commission president José Manuel Barroso proposed that the European Union establish an Institute of Technology with MIT as its model.

Barroso has stumped for the idea in several major speeches, once suggesting that it might be located in Poland, one of the E.U.'s newest members. Although researchers have been largely skeptical, the EIT has gained momentum in some political circles. A group of European Parliament members even suggested a possible campus: their Parliament building in Strasbourg, France—one of two sites where the Parliament sits every month. Many parliamentarians would be happy to give up the building and the trouble of maintaining two home sites.

But on 27 April, the European Research Advisory Board (EURAB), a group of scientists that counsels the commission on policy matters, recommended that it shelve the idea. "As much as we would like to see an EIT come into existence in Europe, we are wary that it cannot be created top-down," the panel says in its statement. "An EIT must grow bottom-up from existing research communities."

Instead, it says, the planned European Research Council (ERC), a body to fund basic research, should be given full support to prompt the kind of competition that helps shape top institutions such as MIT. The ERC—originally proposed by a grass-roots movement of European scientists—was part of the commission's proposal for the €70 billion ($90 billion) 7th Framework program (*Science*, 15 April, p. 342), but its exact funding and structure are still unclear.

E.U. research spokesperson Antonia Mochan says the commission is exploring the EIT proposal. Although it has not ruled out starting a new institution, she says, both research commissioner Janez Potočnik and education commissioner Ján Figeľ have said that perhaps a network of "centers of excellence" across Europe "would be the most relevant way to deal with this issue."

But even such a network worries the advisory panel members. "Our point is that [the institute] would distract from the ERC," says EURAB chair Helga Nowotny of the Vienna Science Center. The panel decided to issue the statement after hearing of increased support for the idea among politicians, she says: "Every science minister from Poland to Portugal wants to host an EIT."

–GRETCHEN VOGEL

CREDIT: PETER CUTTS

GENOMICS

Celera to End Subscriptions and Give Data to Public GenBank

A once-deafening debate over access to human genome sequence data ended quietly last week. Celera Genomics Corp., the company that launched a commercial effort to sequence the human genome and then set about making money from the data, is closing its subscription-based database service and will release its genomic data on humans, rats, and mice to the public.

The move marks the epilogue in the saga of J. Craig Venter, who founded Celera (now owned by Applera Corp.), and Francis Collins, director of the National Human Genome Research Institute in Bethesda, Maryland, and leader of the Human Genome Project, which made its genome sequence data public immediately. The former rivals both praised Celera's move to deposit its data in GenBank. "I think it's a wonderful development. [Applera] deserves a lot of credit for putting this data in the public domain," says Collins. Venter, no longer with Celera, sent an e-mail from his ship, *Sorcerer II*, on a scientific cruise off the coast of Australia, stating that he has been "strongly in favor" of the move, which "sets a good precedent for companies who are sitting on gene and genome data sets that have little or no commercial value but would be of great benefit to the scientific community."

Most scientists would probably say that the outcome was inevitable. "I think the whole model ran its course and was superceded by the public effort," says genome sequencer Richard Gibbs of Baylor University in Waco, Texas.

Four years ago, the race between Collins and Venter to finish a rough draft of the human genome sequence ended in a dead heat. The public effort published its data in *Nature* and deposited them in GenBank, run by the U.S. National Center for Biotechnology Information (NCBI). Celera, whose paper was published in *Science*, shared its data for free only with scientists who agreed not to redistribute or commercialize the data—a restriction that drew loud complaints from many researchers (*Science*, 16 February 2001, p. 1189). The company then created a subscription-based genomic database that later included proprietary data on rats and mice. In early 2002, however, Applera moved the company into drug discovery and Venter left; he now heads his own nonprofit institute.

In its heyday, the Celera Discovery System signed up more than 200 institutions and many drug companies. But subscriptions have fallen off, leading the company to end the service on 1 July and to give 30 billion base pairs of human, mouse, and rat sequence data to GenBank. Making the data public should generate customers for Celera's sister company Applied Biosystems, which supplies researchers with products such as gene expression assays, says Dennis Gilbert, the company's chief scientific officer: "It's a natural evolution of both the business and the science."

Experts say the human data (which includes DNA from Venter and four other people) won't add much new information to the available human sequence. But Celera's mouse and rat data will help publicly funded researchers fill gaps and complete the assembly and validation of the mouse and rat genome sequences. And because Celera and the public efforts sequenced different strains, the data will also help researchers map genetic variation in these model animals.

Come together. Former genome rivals J. Craig Venter (*left*) and Francis Collins (*right*) now see eye to eye on public database.

Two of Celera's remaining subscribers had mixed reactions. Alzheimer's disease researcher Steven Younkin of the Mayo Clinic in Jacksonville, Florida, once viewed Celera's human genome assembly as "a godsend" because its data on gene variants were more reliable than the public assembly's. But Younkin says NCBI's is now just as good.

However, obesity researcher Craig Warden of the University of California, Davis, says his group still uses Celera's mouse genome assembly to check results from the public mouse databases because of its greater accuracy for his genes of interest. "It will be a loss" if GenBank can't catch up, he says.

–JOCELYN KAISER

CREDIT: RICK KOZAK

ScienceScope

Narrowing the Gender Gap

The list of new members of the National Academy of Sciences (NAS) chosen this week contains a record number of women. But the gender ratio—19 women out of the 72 elected—still falls short of the representation of women in most scientific fields.

"As more women get in, more will get elected," predicts California Institute of Technology biologist Alice Huang, a former member of the academies' Committee on Women in Science and Engineering. "The academy realizes that there is something wrong, and they are trying to fix it. But I'm a little surprised at how slow the process is."

This year's class* tops by two the previous high-water mark for academy women, reached in 2003 and 2004, and is a marked increase from the long history of single-digit totals for women. There are now 1976 active NAS members. The academy also chose 18 foreign associates.

The meeting also featured the swan song of NAS President Bruce Albert, whose second 6-year term ends 30 June. The new president is atmospheric chemist Ralph Cicerone, now chancellor of the University of California, Irvine.

–JEFFREY MERVIS

*see nationalacademies.org

Astronomers Want to Be Heard Before NASA Acts

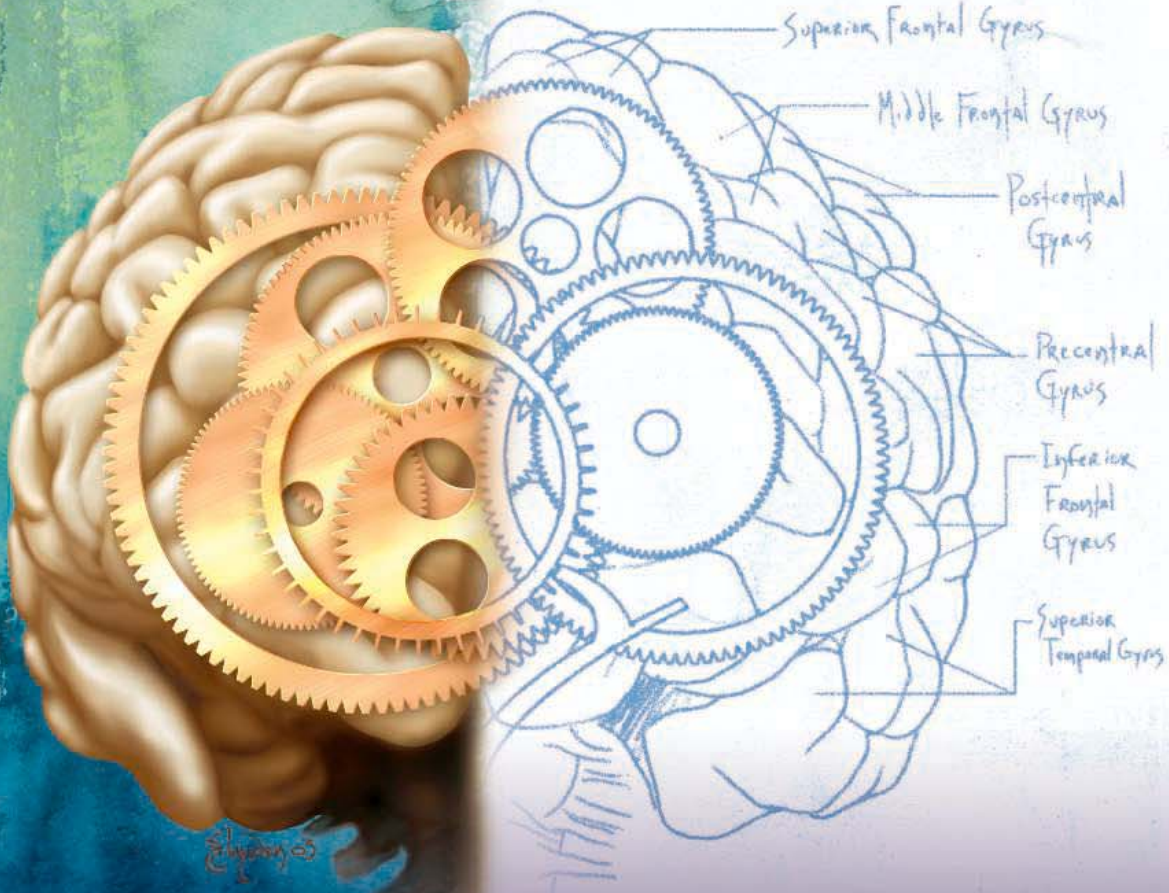
Outside scientists need to weigh in before NASA decides what missions to terminate, says the American Astronomical Society (AAS).

The unusual 2 May statement by the organization, which represents more than 6000 U.S. astronomers and astrophysicists, warns that turning off spacecraft and cutting funds for analyzing spacecraft data—two actions planned to cope with a tight 2005 budget—"can set dangerous precedents for coming years." Continued cuts, says astronomer David Black, who chairs the AAS policy committee, "could put our nation's stature as a leader in space, and the benefits that flow from that leadership, at risk."

The statement calls for NASA to "involve members of the science community in an assessment of missions before finalizing decisions on possible mission terminations." NASA officials say that they will ask for advice on prioritizing missions before taking action this fall—but the final decision, they add, rests with the agency.

–ANDREW LAWLER

IT TAKES BOTH SIDES OF THE BRAIN.



CALL FOR ENTRIES

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ENTRY DEADLINE:
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Awards in each category will be published in the September 23, 2005 issue of Science *and* Science Online *and displayed on the NSF website.*

Accept the challenge. Show how you've mastered the art of understanding.

NASA

U.S. Lawmakers Call for New Earth Science Strategy

Bolstered by a new report from the National Academies, members of the House Science Committee last week attacked the Bush Administration's plans to cancel or delay several missions in NASA's $1.5 billion earth science program. Legislators complained about the lack of a detailed and comprehensive global observation strategy and took issue with NASA's vague plans to transfer some activities to the National Oceanic and Atmospheric Administration (NOAA). Scientists hope the vocal, bipartisan criticism will force NASA to rethink its plans.

"We need a vision and priorities for earth science just as much as we do for exploration and aeronautics," said the committee chair, Representative Sherwood Boehlert (R–NY). Added ranking minority member Representative Bart Gordon (D–TN), "NASA's earth science program faces the prospect of being marginalized."

The National Research Council study (*Science*, 29 April, p. 614) warned that NASA's plans to halt operations of existing satellites, defer or cancel future missions, and reduce funding for analyzing data could undermine an ongoing effort to understand Earth's processes. A proposed $120 million cut for next year would leave the agency's earth science budget $645 million below what the Administration planned just 2 years ago to spend in 2006. NASA is expected to decide next month which of 10 operating satellites should be turned off this year.

Boehlert said NASA's science chief, Al Diaz, told him 1 day before the hearing that the agency planned to transfer some of its responsibilities to NOAA. NASA traditionally has developed advanced instruments and new satellites, whereas NOAA has been in charge of operational systems such as weather satellites. Boehlert and several other lawmakers say they wouldn't object to NOAA's taking on climate observations, but Boehlert is "troubled" by the lack of detail on how and when that would happen and how much it would cost.

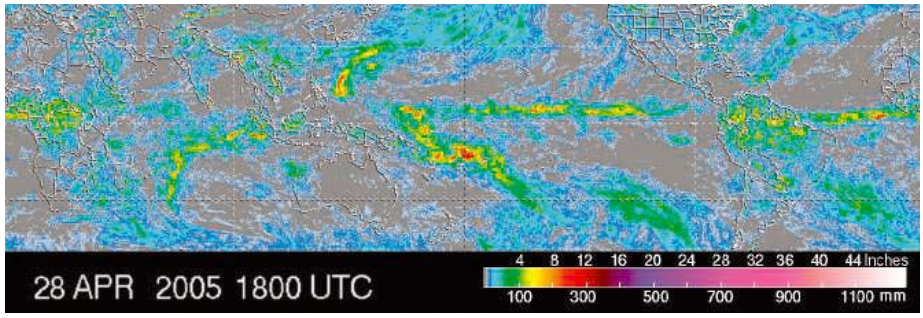
The furor already has prompted NASA to continue work on Glory, a spacecraft designed to study atmospheric aerosols that was axed in the 2006 budget request. Diaz announced the reprieve at the 28 April hearing, adding that he believes the restructuring of the earth science effort would leave the field "much better positioned." The agency has "no intention of abandoning earth science," Diaz says.

Representative Ken Calvert (R–CA) was one of the few legislators to side with Diaz. "I don't think the Administration is trying to hurt earth science," he said. And Representative Dana Rohrabacher (R–CA), a longtime critic of global warming studies, derided the need for "yet another global warming satellite." He added: "When you restructure, … you get rid of things that aren't worthy of the investment."

But those views were not widely shared among the committee. Representative Vernon Ehlers (R–MI) warned Diaz that NOAA would need additional funding to handle any new responsibilities and that Congress needed to be kept in the loop. "This can't be something that is done just because you want to get out from under the financial burden," he said. Any shift would "take a good deal of hard work and coordination—and the concurrence and involvement of both the research community and Congress," he added.

The science committee doesn't control NASA's purse strings, however, and the appropriations panel that does has yet to weigh in on the issue. Still, a pitched battle over the future of NASA's earth science effort seems likely.

—ANDREW LAWLER



CREDIT: TRMM/GSFC/NASA

All wet? Legislators object to NASA's planned cuts to Earth-observing missions like TRMM's monitoring of weekly global rainfall.

DEPARTMENT OF ENERGY

Two-Thirds of Senate Backs More Research

Advocates for the Department of Energy's (DOE's) Office of Science are hoping that a vote of confidence from the U.S. Senate will translate into more money for basic energy research. But a gloomy budget picture may foil their plans.

Last week, 68 senators signed a letter calling for a 3.2% increase for the $3.5 billion DOE office. They want to add $250 million to the Bush Administration's budget request for the 2006 budget year, which begins on 1 October. The letter was circulated by Senators Jeff Bingaman (D–NM) and Lamar Alexander (R–TN), both of whom have large DOE laboratories in their states. This year's effort attracted 13 more signers than a letter circulated last year that opposed a similar Administration cut.

Standing in the way of any boost, however, is a 2006 budget resolution passed last week by both the House and Senate that puts a tight cap on nondefense discretionary spending, the source of all federally funded civilian research. "The appropriators always come back and ask, 'Why didn't you give us more headroom?' " says an aide to Bingaman. The House panel is expected to begin action next week on DOE's 2006 budget.

The senators' letter paints a stark picture of life if the White House's proposed 3.8% cut in DOE science is adopted, including "25% reductions in existing scientific personnel and operations at scientific facilities." It concludes with a warning that "our entire U.S. scientific enterprise is in danger of eroding."

DOE defends its proposed budget as generous given scarce funds, pointing to new monies for nanoscale science and the experimental fusion reactor ITER. Funds added by members for specific projects, says a department spokesperson, disguise the fact that the White House has actually requested a 10% increase over proposed 2005 funding levels.

A concurrent letter-writing campaign in the House has garnered more than 100 signatures, up from 82 last year. Among the new Senate supporters are Democratic budget hawks Russell Feingold (WI) and Kent Conrad (ND). The Senate letter was sent to energy appropriations chair Senator Peter Domenici (R–NM) and ranking member Senator Harry Reid (D–NV). —ELI KINTISCH

Long ignored, the nervous system's glial cells may turn out to be key players in disease and prime targets for therapy

The Dark Side of Glia

When Linda Watkins gave an invited lecture a few years ago, she ruffled the feathers of at least one senior researcher in the audience. Drawing on her studies at the University of Colorado, Boulder, Watkins had argued that nervous system cells called glia contribute to the chronic pain resulting from nerve injury. This was at odds with the predominant thinking in the field, which held that such pain was purely a matter of miscommunication between neurons.

The disapproving researcher, "a big-name person in the pain field whom I respect," Watkins says, wasn't ready to accept that glia were involved. "[He] stood up after my talk and announced in front of the whole audience that he was greatly bothered by my being so glia-centric," she recalls.

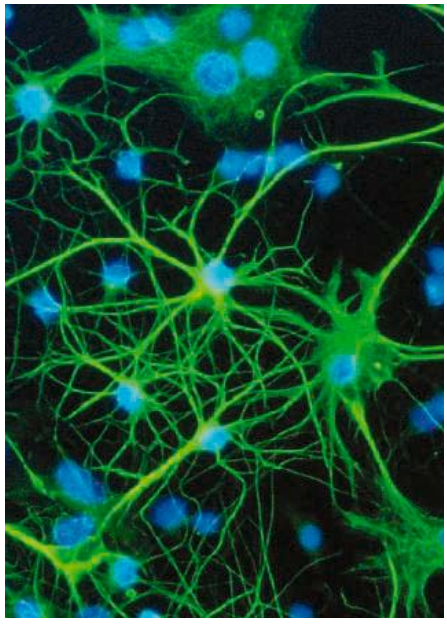
These days such grumblings are becoming more rare. Recent research has shifted the once-heretical view that glia are key players in neuropathic pain into the mainstream. Indeed, on 2 April, the American Pain Society honored Watkins for her contributions to understanding the mechanisms of pain. Other researchers who have recently demonstrated new roles for glia say their work has also begun to garner more attention from colleagues who used to view the cells as mere support staff for the all-important neurons.

The emerging realization of the importance of glia has given new life to an idea that has long lurked at the margins of neuroscience: that glia may have key roles in central nervous system disorders from neuropathic pain and epilepsy to neurodegenerative diseases such as Alzheimer's—and may even contribute to schizophrenia, depression, and other psychiatric disorders. There are also hints that glia may be promising therapeutic targets—a possibility that researchers have scarcely begun to explore.

"We have been very neuron-chauvinistic," concedes Christopher Power, a neurovirologist at the University of Calgary in Canada. "But it's clear [now] that you cannot ignore the roles of glia as important effectors of health and disease."

Workers' revolt

Even the name "glia" reflects the low opinion early neuroanatomists held of these brain cells. It derives from a Greek word meaning "glue," or possibly "slime." Until recently, neuroscientists thought the cells' purpose in life was simply to provide physical support and housekeeping for the neurons, whose electrical impulses underlie all sensation, movement, and thought.



Rising stars. Astrocytes such as these may play key roles in a variety of brain disorders.

In the last decade, however, researchers have discovered that glia, which outnumber neurons by as much as 10 to 1 in some regions of the human brain, have big-time responsibilities. During brain development, they guide migrating neurons to their destinations and instruct them to form the synapses that enable neurons to talk to one another (*Science*, 26 January 2001, pp. 569 and 657). In the adult brain, glia talk back to neurons, releasing neurotransmitters and other signals that regulate the strength of synapses (a possible mechanism of learning). They promote the survival of existing neurons—and perhaps even trigger the birth of new ones.

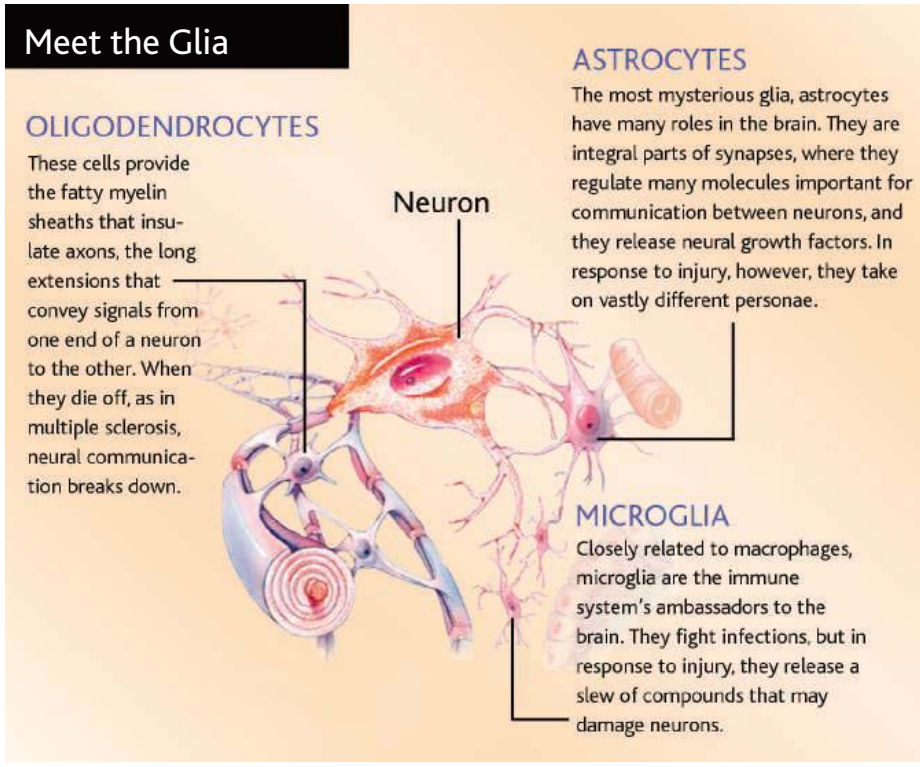
The discovery of all these roles for glia in the healthy brain has prompted researchers to reconsider their connections to diseases. The most clear-cut case of glial involvement in a central nervous system disorder is in multiple sclerosis (MS), one of the most common neurological diseases. Dogma holds that MS is an autoimmune disorder, in which T cells and other immune system cells attack oligodendrocytes, the glia that form a fatty myelin sheath around the axons of neurons in the brain and spinal cord. Axons are neurons' transmission lines, and without insulating myelin, axonal communication breaks down. People with MS suffer movement and balance disruptions as well as impaired vision and other problems.

MS researchers have traditionally considered glia the victims, but there have been hints recently that the story is more complex. A study of tissue from the brainstems and spinal cords of 12 MS patients who died immediately after an outbreak of symptoms, reported by Australian researchers last year in the *Annals of Neurology*, found little evidence of T-cell infiltration into areas of the brain and spinal cord damaged by the disease. Instead, they saw widespread signs that the oligodendrocytes had been self-destructing. To the authors and other MS specialists, the study suggested that the immune reaction long thought to be the root cause of the dis-

ease might be a secondary response to something going awry in the oligodendrocytes.

Last October, Power's team presented another twist on the glia-MS story in *Nature Neuroscience*. Studying brain tissue collected from autopsies of MS patients, the researchers identified a gene that is overactive in astrocytes, another type of glia. The gene, *HERV-W*, jumped from a retrovirus into a primate ancestor of humans about 50 million years ago; its product, a protein called syncytin, now plays an important role in the developing placenta. When the researchers caused the syncytin gene to be overactive in cultured human astrocytes, the cells became toxic to cultured oligodendrocytes. They then inserted the gene into a virus that infects mainly astrocytes and injected the modified virus into the brains of healthy mice; the animals developed MS-like symptoms within 2 weeks and had unusually high numbers of misshapen and dead oligodendrocytes on autopsy.

Astrocytes had not been suspected to play a role in MS, Power says: "We were amazed and really intrigued by this idea that they were involved in the pathogenesis." His team has since developed a compound that blocks expression of the gene in studies with human blood cells and is now testing it in animal models of MS.



Painful truths

The third class of glia, microglia, also appear to have some dirty secrets. They now stand accused of causing neuropathic pain.

Millions of people in the United States suffer from this form of chronic pain as a result of nerve damage caused by physical injury, surgery, viral infection, or chemotherapy (*Science*, 16 July 2004, p. 326). For many afflicted people, even the gentle brush of clothing against the skin can be excruciating.

Morphine and other pain drugs don't help most people with neuropathic pain. Watkins suspects that current drugs are ineffective because they are intended to work on neurons, whereas increasing evidence suggests that microglia are the real instigators. When a healthy person steps on a tack, pain-sensitive neurons in the foot send a message to neurons in the spinal cord, which relay the message up to the brain. In people with neuropathic pain, the pain-sensing neurons become hyperexcited. In effect, they scream at the spinal cord neurons instead of talking to them in a normal voice—even when there's no tack or other painful stimulus.

In the 1970s, researchers discovered that the types of injuries that lead to chronic pain trigger microglial cells in the spinal cord to proliferate and spew out various signaling molecules. But until recently it wasn't clear whether this glial activation somehow caused the sensory neurons' excitability or was merely an unrelated side effect, says Michael Salter, a neuroscientist at the University of Toronto in Canada.

A 2003 study in *Nature* provided strong evidence that activated microglia are the cause. Salter, Kazuhide Inoue of the National Institute of Health Sciences in Tokyo, and their colleagues identified a protein that is necessary for neuropathic pain in rats with a severed nerve and showed that this cell surface receptor is displayed only by activated microglia. Much as a typical rat withdraws quickly from a painfully hot surface, the rats with a severed nerve withdraw a paw in response to a light touch. But when the team blocked the microglia receptor called $P2X_4$ with a drug injected into the spinal column, the behavior of the injured rats rapidly returned to normal. When the drug wore off, the abnormal pain responses returned.

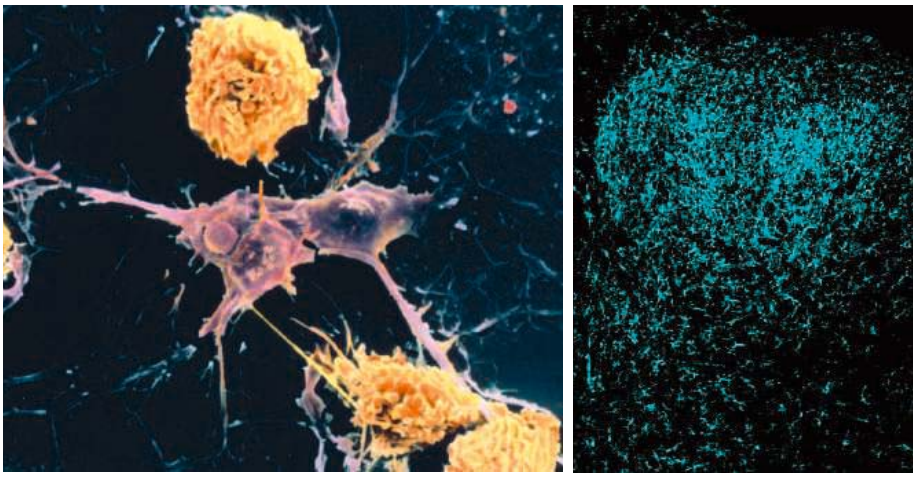
> "We have been very neuron-chauvinistic, but it's clear that you cannot ignore the roles of glia as important effectors of health and disease."
> —CHRISTOPHER POWER, UNIVERSITY OF CALGARY

Two big mysteries now confront researchers investigating the link between glia and neuropathic pain, Salter says. One puzzle is what activates microglia after injury. The other is how activated microglia hypersensitize sensory neurons in the spinal cord. Solving either one could have huge clinical payoffs in terms of treating pain.

A paper published online 4 April in the *Proceedings of the National Academy of Sciences* provides an important clue, pointing to a possible trigger of microglial activation. Joyce DeLeo and colleagues at Dartmouth Medical School in Hanover, New Hampshire, had previously found that nerve injury activates a receptor called Toll-like receptor 4 (TLR4), which in the central nervous system is only expressed on microglia. In the new study, the researchers found that genetically altered mice lacking TLR4 showed markedly reduced microglial activation after nerve injury, as well as reduced hypersensitivity to pain. Blocking expression of the receptor in normal rats prior to a nerve injury yielded similar results.

"It's a beautiful series of experiments," says Watkins. She and Salter point out, however, that TLR4 is likely only one of several routes for microglial activation after nerve injury. Watkins's group, for example, has identified another candidate trigger—a protein called fractalkine—expressed on the surface of neurons.

Much of the research on the second mystery—how microglia excite sensory neurons—has focused on immune system messengers called proinflammatory cytokines. Many researchers have suggested that proinflammatory cytokines secreted by activated microglia increase neural excitability and increase sensitivity to pain when injected into



Glial woes. The death of oligodendrocytes (*left*, purple) spells trouble for multiple sclerosis patients. When activated, microglia (*right*, blue) in the spinal cord may trigger chronic pain.

the spinal cords of rats. Despite this promise, however, few cytokine-blocking drugs have made it to human trials, largely because of their potent immunosuppressive action and other side effects.

The potential pain therapy that most excites Watkins involves a cytokine from microglia that dampens inflammatory responses. In work described at recent conferences, she and colleagues delivered naked DNA for this protein, interleukin-10 (IL-10), to the spinal cords of rats with nerve damage. The cells surrounding the cords took up the DNA and bathed nearby glia in IL-10. The gene-delivery procedure involves something like a reverse spinal tap and appears to relieve neuropathic pain in the rodents for at least 3 months. "They behave like normal animals," says Watkins. Her team is now collaborating with Avigen, a biotech company in Alameda, California, in hopes of gaining Food and Drug Administration (FDA) approval for a clinical trial for neuropathic pain patients.

Watkins and her colleagues have also begun testing the gene-therapy technique in a rat model of chemotherapy-induced pain. Many cancer patients, notes Watkins, would rather forgo potentially lifesaving drugs than suffer the pain that comes with them. "People are literally dying because of the pain caused by chemotherapy," she says.

Too much excitement

The ability of glia to make nerve cells hyperactive may also play a role in epilepsy, another disorder long thought to be purely a problem with neurons. Raimondo D'Ambrosio, a neuroscientist at the University of Washington, Seattle, has been studying the role of glia in trauma-induced epilepsy, which occurs in about half the people who suffer the most severe head injuries. According to him, a factor in such epilepsy is the split personalities of astrocytes, which together with microglia become activated in response to injury.

Many researchers describe astrocyte activation as a Jekyll-to-Hyde transition. Quiescent astrocytes have long, armlike extensions that wrap around synapses and allow them to regulate the concentrations of ions and neurotransmitters around these nerve cell junctions. But in response to a head injury or other trauma, astrocytes often withdraw their arms and slack off on their stabilizing chores. Some of them migrate to the site of injury, where they help repair the damaged area and create a protective scar. This emergency response may ultimately be beneficial, but it causes problems too.

"Reactive astrocytes are not as good as normal astrocytes at taking care of brain physiology," D'Ambrosio says. His team has found that after head trauma, astrocytes reduce activity of the protein channels that allow them to draw potassium out of the space around neurons. As a result, potassium builds up in the extracellular space, making the neurons more likely to fire in synchronous patterns typical of epilepsy. "There's no question that excess extracellular potassium facilitates seizures," D'Ambrosio says.

> "Many of the drugs we already have might conceivably work on glia, and people just haven't realized it yet."
>
> —BEN BARRES, STANFORD UNIVERSITY

Other changes in activated glia may also contribute to the neural excitability underlying seizures. Astrocytes recycle the neurotransmitter glutamate. Once glutamate is released at a synapse, it excites neurons until it is removed. Astrocytes handle about 90% of this glutamate clearance, but activated or injured astrocytes may abandon this task and may even release glutamate themselves.

Furthermore, as in neuropathic pain, cytokines released by activated glial cells may contribute to epilepsy, D'Ambrosio says. Work by other researchers has shown that levels of some cytokines spike in the cerebrospinal fluid of people and in the brains of rats after a seizure. When glial cells are genetically engineered to overproduce certain proinflammatory cytokines in mice, the animals become more prone to seizures.

D'Ambrosio also suspects that glia play important roles in more common types of epilepsy that aren't caused by trauma. "Glia can affect nearly every aspect of neuronal excitability and function," he says, which makes them possible therapeutic targets for any form of epilepsy.

Falling apart

There's a growing suspicion that misguided glia can do more than overexcite neurons—they may even kill them.

One of the first clues that glia may be involved in neurodegenerative disorders came from studies on a form of dementia that afflicts 10% to 20% of those infected with HIV. The virus's target of choice in the brain is microglia; it infects neurons very rarely, if at all.

How infected microglia conspire to kill off neurons and cause dementia is not known. One possibility is that inflammatory cytokines and other compounds released by microglia injure neurons directly; another is that the microglia activate astrocytes, which abandon their glutamate-recycling duties, allowing the neurotransmitter to build up and kill neurons by overexciting them.

Both mechanisms may also be at work in a wide range of neurodegenerative disorders, says Robert Nagele, who studies Alzheimer's disease at the University of Medicine and Dentistry of New Jersey in Stratford. For example, activated microglia invade the amyloid plaques in the brain that are the hallmark of Alzheimer's disease. Activated astrocytes also form a halo around the plaques. Many researchers agree that the inflammatory glial response contributes to the damage seen in Alzheimer's brains, says Nagele, but exactly how is a matter of debate.

"There's a long list of [brain] diseases that are now appreciated to have an inflammatory component," says Gary Landreth, an Alzheimer's disease researcher at Case Western Reserve University in Cleveland, Ohio. Although many anti-inflammatory drugs are being tested for Alzheimer's disease and other neurodegenerative disorders, the results have been mixed. These drugs probably reduce neurodegeneration in part by inhibiting the inflammatory response of glia, Landreth says, but they

CREDITS (LEFT TO RIGHT): JOHN ZAJICEK/PHOTO RESEARCHERS INC.; MAKORO TSUDA

act throughout the body. A drug that targeted glia specifically might be very valuable, he says, if it dampened inflammation in the brain without weakening the immune system—but so far, no such compounds have been developed.

Tackling glutamate excitotoxicity has also been tricky. The drug memantine, which is intended to protect neurons from this threat and was approved in the United States in 2003 for Alzheimer's disease, modestly slows cognitive decline in patients but doesn't seem to thwart the brain's eventual neurodegeneration. Although memantine blocks a type of glutamate receptor on neurons, a paper published in January in *Nature* suggests another way to prevent excitotoxicity: boosting the activity of the glutamate transporter on astrocytes, the molecular pump responsible for clearing glutamate from the synapse.

Jeffrey Rothstein of Johns Hopkins University School of Medicine in Baltimore, Maryland, and colleagues screened more than 1000 FDA-approved drugs and discovered that a class of widely used antibiotics, the so-called β-lactam antibiotics, which includes penicillin and its derivatives, spurs astrocytes' production of glutamate transporters and increases the glial cells' uptake of glutamate. In a mouse model of the fatal neurodegenerative disease amyotrophic lateral sclerosis, one of these antibiotics delayed neuron loss and prolonged survival.

On 22 February, the European Commission approved a drug that appears to work primarily on glia for Parkinson's disease, another neurodegenerative disorder. The drug rasagiline is already on the market for Parkinson's disease in Israel and is under consideration by FDA for use in the United States. The drug inhibits the monoamine oxidase B enzyme, which is found predominantly in microglia and astrocytes, says its inventor Moussa Youdim, a pharmacologist at Technion–Israel Institute of Technology in Haifa.

Rasagiline was thought to work largely by preventing the enzyme from breaking down the neurotransmitter dopamine, which is deficient in Parkinson's patients. But the drug also appears to have glia-based neuroprotective effects, Youdim says. His team reported in February in the journal *Mechanisms of Ageing and Development* that the drug and related compounds sop up iron, preventing the metal from building up inside glia and undergoing chemical reactions that create dangerous free radical compounds that can seep out and wreak havoc on neurons. Researchers suspect that this process plays a role in other neurodegenerative disorders, and Youdim and colleagues are now testing rasagiline—as well as related compounds they've created recently that are even more effective at binding iron—in animal models of Alzheimer's and Huntington's disease.

More than glue

The most speculative links between glia and human disease concern psychiatric disorders such as schizophrenia and depression. Postmortem studies of patients with schizophrenia, bipolar disorder, and depression have turned up oddities in the numbers of glia in brain regions implicated in those disorders. In 1999, for example, Grazyna Rajkowska of the University of Mississippi Medical Center in Jackson and colleagues reported that people with major depression had at the time of their death low glial cell counts in certain areas of the frontal cortex, including regions thought to be important for cognition, mood, and motivation.

Since then, Rajkowska's lab has found evidence that AWOL astrocytes account for those low glial counts and that astrocyte counts are particularly low in depressed people who die young (often by suicide). Because astrocytes help stabilize the environment for neurons and provide them with growth factors, Rajkowska speculates that an early deficit in astrocytes could debilitate neural circuits in brain areas involved in regulating mood. "I believe everything starts with glial pathology," she says.

In other postmortem studies, Joseph Price, a neuroanatomist at Washington University in St. Louis, Missouri, and colleagues have found deficits in oligodendrocytes in the frontal cortex of depressed patients. Last year they reported a 20% to 30% reduction in the number of oligodendrocytes in the amygdala, a key emotion center. Price speculates that because oligodendrocytes provide the critical insulation on axons, the deficits could contribute to depression by causing faulty wiring in mood-related brain areas.

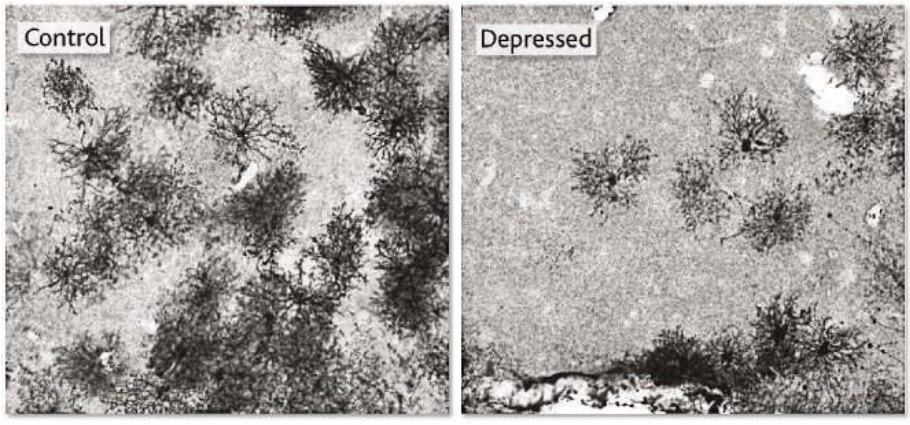
A DNA microarray study published in the March issue of *Molecular Psychiatry* further implicates oligodendrocyte abnormalities in depression. A team from Wyeth Research in Princeton, New Jersey, and the National Institute on Drug Abuse in Baltimore, Maryland, found that the activity of 17 genes related to oligodendrocyte functions including myelination and cell communication were altered in postmortem temporal cortex tissue of patients with major depressive disorder.

"So far, the data look quite intriguing in terms of glia in postmortem brains," says psychiatrist Husseini Manji of the National Institute of Mental Health in Bethesda, Maryland. But he adds that at this point, hypotheses about how glial deficits might lead to psychiatric symptoms are "very preliminary."

That's not to say researchers lack theories. Astrocytes control the amount of glutamate at synapses, and abnormalities in levels of this neurotransmitter have been tied to a variety of psychiatric disorders, including depression, anxiety, and schizophrenia (*Science*, 20 June 2003, p. 1866). Astrocytes also recycle other neurotransmitters implicated in psychiatric disorders. In the last few years, a research team led by Masato Inazu of Tokyo Medical University in Japan has identified several transporters on astrocytes that take up serotonin, dopamine, and other so-called monoamine neurotransmitters. Many existing psychiatric drugs tweak levels of these neurotransmitters, and Inazu and colleagues have found that some antidepressant drugs alter activity of those astrocyte transporters. "Many of the drugs we already have might conceivably work on glia, and people just haven't realized it yet," says Ben Barres, a glial biologist at Stanford University in California.

Given the fairly modest healing abilities of current neuropsychiatric drugs, researchers think the glial leads are well worth pursuing. "There are many more glial cells in the brain than there are neurons, and they're not just glue," says Robert Schwarcz, a pharmacologist at the Maryland Psychiatric Research Center in Baltimore. "They're actively participating in many important functions, and if anything goes wrong with them, there's going to be dysfunction and disease."

–GREG MILLER



Suspicious disappearance. The loss of astrocytes (dark masses) may contribute to depression.

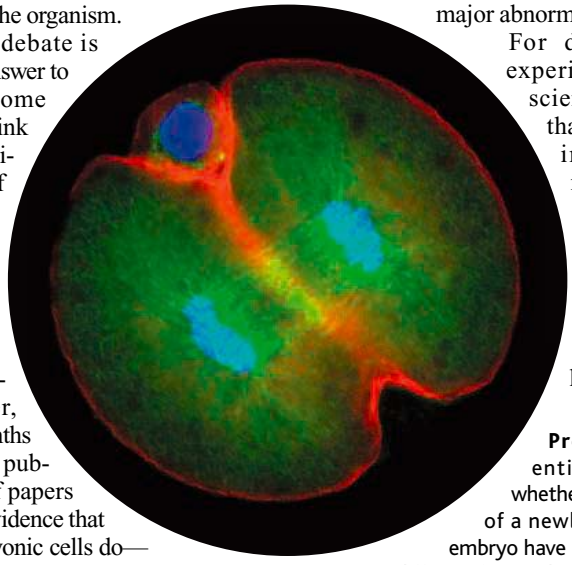
CREDIT: G. RAJKOWSKA AND J. J. MIGUEL-HIDALGO/UNIVERSITY OF MISSISSIPPI MEDICAL CETNER

Embryologists Polarized Over Early Cell Fate Determination

Scientists are trying to determine when the first asymmetry occurs in the mouse embryo, but the embryo has so far thwarted their efforts

Embryologist Hans Spemann famously pointed out 60 years ago that we are standing and walking with parts of our body that would have been used for thinking had they developed in another part of the embryo. Yet scientists still aren't sure when the cells in a mammalian embryo start to take on the individual identities that will determine their eventual fates in the organism.

Currently, a debate is raging over the answer to this question. Some embryologists think that even the earliest cells have, if not an immutable destiny, at least a tendency to form one part of the embryo or another. Not everyone is convinced, however, and in recent months researchers have published a flurry of papers laying out their evidence that the earliest embryonic cells do—or don't—carry inherent preferences that tilt them toward one destiny or another.



Predestined? Scientists are debating whether the first two cells of a newly created mouse embryo have a tendency toward different fates in later development.

The studies address one of developmental biologists' most fundamental questions: How can a single cell—the fertilized egg—give rise to embryos and later animals with a distinct front, back, top, and bottom? In some species, the answers are well known. The unfertilized fly egg, for example, already contains concentrations of proteins in different regions that influence the eventual location of the fly's head and posterior. In frogs, one of the first events after fertilization is the development of a prominent "gray crescent" on the side of the egg opposite where the sperm has just entered, which contains key signals crucial for development.

But pinning down what happens in the mammalian embryo has always been much more difficult. In the first place, the eggs of mammals are tiny—less than one-thousandth of the volume of a frog egg. And their embryos inconveniently develop inside the mother's body, making direct observations in the embryo's natural environment extremely difficult. What is certain is that the cells of mammalian embryos are much more flexible than those of their amphibian or insect counterparts. Scientists can take a two-, four-, or even eight-cell mouse embryo, tease the cells apart, recombine them with cells from another embryo, and produce a healthy mouse. In frogs and fish, such tricks yield animals with two heads or other major abnormalities.

For decades, those experiments led most scientists to assume that the cells making up an early mouse embryo are equivalent, and that the first signs of embryonic polarity—having an up-down or left-right axis—appear in the blastocyst, a slightly oblong ball of a few dozen cells that forms about a week after fertilization. "The paradigm has been that [the mouse embryo] is a blank sheet until you start to make the blastocyst," says developmental biologist Janet Rossant of the Samuel Lunenfeld Institute at the University of Toronto, Canada.

By that time, cells have developed into at least two types: those of the inner cell mass, which will form the fetus as well as parts of the placenta and surrounding tissues, and the trophoblast cells, which will form much of the placenta but will not contribute to the developing fetus. At this stage, the embryo has a clear polarity: The inner cell mass clusters at one end of the blastocyst, which developmental biologists call the embryonic side, and the other half, called the abembryonic side, contains a hollow cavity called the blastocoel.

But over the past decade, several groups probing the embryo's earliest stages have found evidence suggesting that the embryo's directionality might arise well before blastocyst formation. Some of the first hints came from Richard Gardner's lab at the University of Oxford in the United Kingdom. In 2001, he and his colleagues reported experiments in which they used tiny drops of oil to mark the two cells produced by the first division of the fertilized mouse egg. The researchers found evidence that the "equator" created by the first cell division tends to be roughly in the same plane as to the equator dividing the embryonic and abembryonic regions of the blastocyst, leading them to wonder if the egg itself might have north and south poles that influence the fate of cells derived from one hemisphere or the other. "It took 5 years to publish because I didn't believe it myself," Gardner says.

At about the same time, Magdalena Zernicka-Goetz and her colleagues at the University of Cambridge, U.K., started to look at exactly where the progeny of the embryo's first two cells end up. In 2001, they reported in *Development* that by carefully marking the sister cells with different-colored dyes, they found that the descendents of one tend to form the embryonic side—whereas the other gave rise to the abembryonic side. That suggested, the authors said, that from the first division on, the embryo has a polarity of its own.

Later that year, the team reported results from a complex and marathonlike set of experiments to see if cells in the four-cell-stage embryo are distinguishable from one another. To identify the four cells reproducibly, the researchers took advantage of the fact that the mouse egg itself is not perfectly symmetrical. It remains attached to the so-called second polar body, which contains genetic material ejected during the egg's maturation. The side with the polar body is called the "animal" pole, whereas the opposite side is called the "vegetal" pole.

According to Zernicka-Goetz and her colleagues, the two cells formed by the first cell division almost always divide with their cleavage planes roughly perpendicular to each other. One division follows the longitude of the oocyte and the other the latitude, so that the four-cell embryo consists of one cell containing mainly animal cytoplasm, one with mostly vegetal cytoplasm, and two cells containing a mix of animal and vegetal cytoplasm.

In experiments that began at 6 a.m. and ran for nearly 20 hours, Zernicka-Goetz, Karolina Piotrowska-Nitsche, also at Cambridge, and their colleagues carefully tracked the divisions of early embryos, broke them apart at the four-cell stage, and created embryo chimeras with known compositions of the different cells. In previous

CREDIT: T. HIRAGI AND D. SOLTER, *NATURE* **430**, 360 (2004)

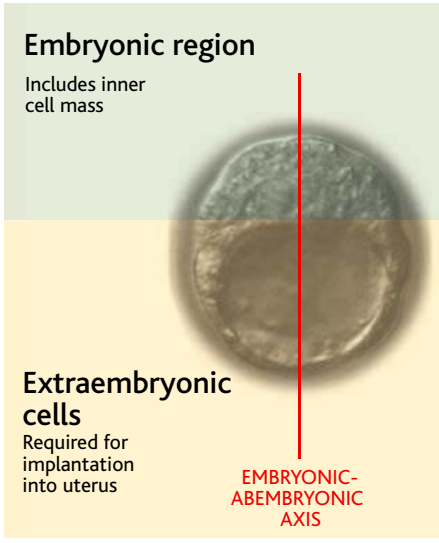
experiments in which chimeric embryos were created by randomly mixing cells from four-cell embryos, nearly all the chimeras developed normally.

In contrast, the Cambridge team found that none of the chimeras made from cells that contained predominantly vegetal cytoplasm developed when implanted into foster mothers. Chimeras containing predominantly animal cytoplasm developed about 25% of the time, and those containing cytoplasm from both hemispheres developed 87% of the time—a result comparable to that of the random mixing experiments.

"The result shocked us," says Zernicka-Goetz. "It certainly isn't what we expected." But she says further studies support the result. Last month, she and Piotrowska-Nitsche reported in *Mechanisms of Development* that the predominantly "vegetal" cell of the four-cell embryo contributes almost nothing to the inner cell mass of the blastocyst.

None of the rules her team has identified are hard and fast, Zernicka-Goetz admits. And she acknowledges that many scientists continue to believe that because early cells are so flexible, there is no underlying pattern in the egg or early embryo. However, she says, "the other possibility is that there is a pattern, but not a determinant pattern—that you have a set of biases that push cells toward a certain path. We think we have evidence that this is closer to the truth."

Davor Solter of the Max Planck Institute for Immunobiology in Freiburg, Germany, is one of those who is not yet convinced. Indeed, he disputes most of Zernicka-Goetz's conclusions. For one, he and his colleague Takashi Hiiragi, also at the Max Planck Institute for Immunobiology, find no tendency for the first cell division to occur on a plane perpendicular to the animal-vegetal equator. In contrast, they reported in *Nature* last year that in their time-lapse recordings of the first cell division, the angle of division is mostly influenced by the relative location of the sperm and oocyte pronuclei as they move toward the center of the cell. They claim that the polar body, which starts out marking the so-called animal pole, actually moves toward the cleavage plane in about half of the embryos they observed, which might have influenced the observations that Gardner and Zernicka-Goetz report.



Early patterns. The blastocyst-stage embryo has distinct embryonic and abembryonic poles.

And for another, Solter and Hiiragi don't see evidence that the first cell division correlates with the later axis of the blastocyst. One factor that might be complicating the experiments is the dynamic movements of the embryos as they develop. Solter says that "these embryos are like spinning yo-yos" in the time-lapse movies, which makes it nearly impossible to keep track of the angle of the original cell divisions.

Solter, Hiiragi, and several colleagues also report in the 1 May issue of *Genes and Development* that they can find no evidence that one sister cell contributes preferentially to one end of the blastocyst or the other. In these experiments, the team used dye techniques similar to those of Zernicka-Goetz to mark cells at the two-cell stage and then filmed embryos using time-lapse photography for 3 days as the two cells grew into blastocysts. But the results showed no clear pattern of daughter cells in the resulting blastocysts, Solter says.

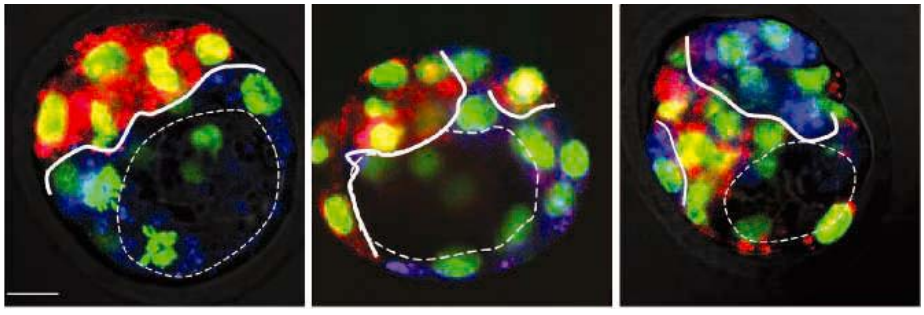
Some of the blastocysts did show patterns similar to those Zernicka-Goetz reports, Solter acknowledges, but they were a minority. Only about 25% of the embryos they observed had blastocysts in which the daughter cells sorted predominantly into the embryonic or abembryonic part—far less than the nearly 70% reported by Zernicka-Goetz and other groups. Solter, Hiiragi, and their colleagues propose that mechanical forces on and within the developing embryo determine its eventual polarity. But Zernicka-Goetz says her team used a different method of measuring the boundary between the cell types, by painstakingly counting cells at different layers in the blastocyst. "I would be very interested to ask them to analyze their data in the same way," she says. "Only then can we really compare our results."

For his part, Gardner is taking something of a middle ground. "There is undeniable evidence that there is prepatterning" in the embryo, he says. But different techniques used in different labs—and the inherent flexibility of the embryo itself—make it very difficult to determine exactly how much. For example, he says, his lab, like that of Zernicka-Goetz, continues to see a consistent pattern between the plane of the first cell division and the shape of the blastocyst. But, Gardner adds, in his lab "we don't see a shred of evidence" that the cells of the four-cell embryo are different.

If there is one point on which all parties would agree, it's that the techniques used so far make clear answers extremely hard to come by. "When people observe embryos, there's always a lot of variability," says the Lunenfeld Institute's Rossant. "If you're looking for a certain result, you'll see it, but there will always be some results that do not fit." So far, she says, "you have to say that arguments on both sides are inconclusive."

The definitive experiment, Rossant and others say, would be to identify a gene or protein, like those already identified in frog or fly embryos, that clearly marks the fate of different early embryonic cells. Zernicka-Goetz and her colleagues are searching for such a factor, she says, looking for differences in gene expression signatures, or in more subtle modifications of the cell's internal architecture. If the genetic search is successful, Solter says, he will be convinced. If someone could find a gene expressed in a specific region of the egg—or in one early cell and not others—and if removing that gene interrupts development, "then absolutely, prepatterning is proven," Solter says. "If such a gene exists, it will be found."

–GRETCHEN VOGEL



Axis of disagreement. In some experiments in which one cell of the two-cell embryo is stained red and the other blue, the progeny of one cell tends to form the embryonic region of the blastocyst, whereas the other gives rise to the abembryonic region (*micrograph on left*). In other embryos (*middle and right*), the line is more difficult to draw. (DNA is stained green.)

CREDITS (TOP TO BOTTOM): ADAPTED FROM R. PEDERSEN, *NATURE* **409**, 473 (2001); PHOTO FROM T. HIIRAGI; B. PLUSA *ET AL.*, *NATURE* **434**, 391 (2005)

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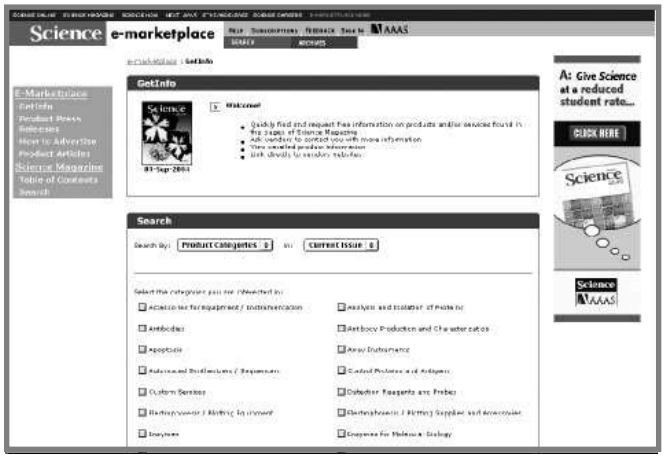

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Electronic Paper: A Revolution About to Unfold?

Developers have high hopes for paper-thin flexible displays, but some technologists say "killer apps" to drive the technology remain to be found

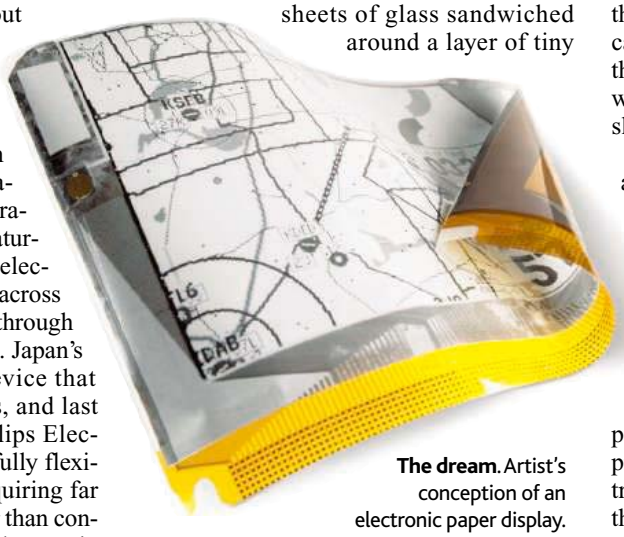
In an exhibition hall at the 2005 World Exposition in Aichi, Japan, a gigantic newspaper covering more than 5 square meters delivers the news to passersby in crisp black and white. Unlike a traditional broadsheet, which goes from printing press to trash bin within a few hours, the Yomiuir Global Newspaper never becomes old news. Instead, the display rewrites itself electronically twice a day, keeping readers up-to-date without generating wastepaper. But the real message is the medium itself: Electronic paper is coming.

Produced by Japan's TOPPAN Printing Co. and the American high-tech firm E Ink, the newspaper is just one of several demonstrations, prototypes, and products featuring the new technology. Already, electronic paper signs direct students across college campuses and passengers through the East Railway Station in Berlin. Japan's Sony Corp. sells a tabletlike device that stores and displays whole books, and last year the Netherlands' Royal Philips Electronics unveiled a prototype of a fully flexible sheet of electronic paper. Requiring far less power and potentially cheaper than conventional flat-panel displays, electronic paper could change how consumers view the world. But even as the budding technology emerges from the laboratory, technologists are debating a crucial question: Is there really a need for electronic paper?

"The problem is that nobody can think of anything new that genuinely needs the flexibility," says Kim Allen, an industry analyst with iSuppli Corp. in Santa Clara, California. "All the things that are technically possible are well served by rigid displays." But Michael McCreary, a chemist and vice president for research and advanced development at E Ink in Cambridge, Massachusetts, says electronic paper will be so versatile it will create its own need. "It's going to do things that cannot be done with either paper or displays today," he says. "I think that this is going to create a revolution." Developers envision smart signs and high-tech scrolls that relegate newspapers and books to the recycling bin of history. Still, they must overcome several stiff technological challenges before pliable electronic paper is ready for mass production. And neither book publishers nor bookies know which applications of electronic paper are sure bets or how they'll pay off.

Electric ink and organic circuits

In spite of its name, electronic paper is the technological cousin of the flat-panel computer screen. A computer's liquid crystal display (LCD) consists of two sheets of glass sandwiched around a layer of tiny transistors and a layer of liquid crystal material—a soup of rodlike molecules that snuggle together a bit like sardines in a can. The screen is illuminated from behind, and ordinarily the molecules let light pass through them. But when a transistor applies a voltage to a point on the screen, or pixel, the molecules rearrange themselves to block the light and darken the pixel. Filters tint the light from neighboring pixels red, blue, or green to create a color image.



The dream. Artist's conception of an electronic paper display.

In electronic paper, too, each pixel changes color when a voltage is applied. But instead of emitting light, electronic paper merely reflects it, dramatically reducing power consumption. A pixel in electronic paper also holds its color without voltage. Thanks to such "bistability," electronic paper uses power only when the image on it changes. All told, electronic paper may use less than 1/10,000 as much power as a computer's LCD.

Researchers are developing several different technologies for the color-changing electronic ink. E Ink's "electrophoretic" material consists of microcapsules embedded in a plastic sheet, each filled with a clear liquid and submicrometer-sized particles, some colored black and others colored white. The black and white particles carry opposite electric charges; a pulse of voltage from the underlying transistor can make the white ones rise toward the surface of the display and the black ones sink away from it, or vice versa. Gyricon, a subsidiary of Xerox Corp. based in Ann Arbor, Michigan, does a similar trick with plastic spheres about 100 micrometers wide, each half black and half white with opposite electrical charges on the two sides. The spheres simply flip in response to a pulse of voltage. E Ink's and Gyricon's products provide better contrast than a computer's or cell phone's LCD and can be read even in direct sunlight. But for the moment, both are essentially black-and-white technologies that switch pixels too slowly to show moving images.

Others are taking different tacks. NTERA, a high-tech company based in Dublin, Ireland, makes bistable displays that employ "electrochromic" dyes: molecules that switch from transparent to specific colors when they absorb electric charge. In contrast, Kent Displays Inc. in Kent, Ohio, has developed a reflective LCD that is bistable.

Creating a truly paperlike, flexible display poses equally challenging technical problems. The problem lies behind the electronic ink, in the "backplane" of transistors that activate it. That's because the crystalline silicon from which transistors are usually made is brittle and must be deposited on a stiff substrate such as glass.

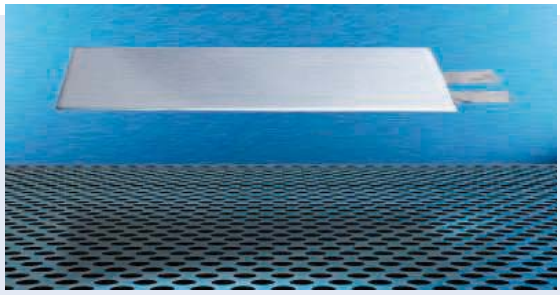
To make a flexible backplane, researchers are developing materials such as non crystalline amorphous silicon, says Zhenan Bao, a chemist at Stanford University in California. Perhaps most promising, she says, are plastics that have electrical properties similar to those of silicon. Such "organic semiconductors" bend easily and can be deposited on flimsy plastic substrates. And in theory, Bao says, a simple inkjet printer could lay down organic circuits much more cheaply than the complicated multistep process now used to etch circuits into silicon.

Philips and E Ink have teamed up to make a flexible electronic paper display driven by organic semiconductors. The low-resolution screen, roughly as thick as a sheet of printer paper, measures 12.7 centimeters along the diagonal and rolls up into a tube 1.5 centimeters wide. Philips envisions using the screens for retractable displays on hand-held devices and plans to begin design for pro-

CREDIT: COURTESY OF POLYMER VISION

Shrinking Dimensions Spur Research Into Ever-Slimmer Batteries

As electronic devices shrink toward paper-thinness, researchers and companies around the globe are scrambling to come up with novel materials and designs for two-dimensional batteries to power them.



Never too thin. Spray-on techniques could make "flat" power sources like this Solicore battery look positively chunky.

Like their 3D cousins, so-called flat batteries convert chemical energy to electricity by making an electrical connection between a negative electrode, or anode, and a positively charged electrode, called the cathode. Electrons flow from the cathode to the anode through a conductive material, known as an electrolyte. In flat batteries, however, each layer is often mere micrometers thick.

Companies already sell a variety of rechargeable and nonrechargeable flat batteries. Most serve low-power applications such as electronic ID tags and "smart" credit cards sporting tiny displays. To increase the output appreciably, technologists would have to spread the batteries out over much larger areas—a move that would either leave them thin and flimsy or require bulky, heavy shielding.

To get around this problem, Larry Dubois, a chemist at SRI International in Menlo Park, California, and colleagues are working on a couple of possible fixes. First, Dubois's team has developed a scheme for printing multiple thin batteries atop one another. The setup uses a device resembling a computer-controlled ink-jet printer to lay down successive layers of liquid precursors for the electrodes and electrolytes of a rechargeable lithium-ion battery. The setup makes it relatively straightforward to layer multiple thin cells to improve both the total amount of power they can hold and the maximum voltage they can supply. The battery is then encased in a plastic housing. Because printing technology tends to be far cheaper than the traditional vacuum-deposition techniques used in battery manufacturing, the new technique could sharply reduce the high cost of lithium batteries. The computer can also spray down batteries to fit any shape. "We've even written the text of the Declaration of Independence" as a battery, Dubois says.

In a second twist, Dubois's team has recently created batteries coated atop thin fiber-based electrolytes. The work is at an early stage, but the researchers hope to create batteries that can be integrated directly onto structural materials, such as the plastic fibers in a laptop case. That way the material that provides structural support for the computer could power it as well.

–ROBERT F. SERVICE

duction this year, says Hans Driessen, a spokesperson for Philips Research in Eindhoven, Netherlands. Philips guarantees the screen will roll and unroll at least 2000 times before it conks out—which may not be enough for people who make 10 calls per day from their cell phones.

To market, to market ...

Electronic paper may someday rewrite the concept of the book, but to bring the technology to market, developers are using it first to spruce up the humble store sign. Gyricon sells a wirelessly programmable sign for $1295 and has developed a system to control multiple signs from a central location. "The reason retailers do a 3-day sale is it takes a day to put up the signs and a day to take them back down," says Jim Welch, director of marketing and communications at Gyricon. "This opens the way for having an instant sale." However, systems using smaller LCD shelf labels already exist.

Developers also hope to produce larger, lower-power, and easier-to-read displays for cell phones and other hand-held devices. E Ink has positioned itself to sell its electronic ink by the sheet as a commodity to electronics manufacturers. The company already supplies the displays for Sony's LIBRIé electronic book reader. Introduced last year and sold only in Japan, the LIBRIé weighs 300 grams, holds 10 megabytes of text, and can flip more than 10,000 virtual pages before draining its four AAA batteries. Still, the book reader looks less like a new type of paper than a black-and-white subspecies of personal digital assistant.

Ultimately, developers envision a kind of smart scroll that downloads newspapers, magazines, and books wirelessly, says Nick Sheridon, a physicist at the Palo Alto Research Center in California and inventor of Gyricon's technology. Sheridon doubts electronic paper will ever entirely replace paper. "There are books that I will always want on paper," Sheridon says. "But I don't think that anyone is so attached to their newspaper." If it lives up to its promise, electronic paper could slash newspaper companies' printing and distribution costs, says George Irish, president of Hearst Newspapers in New York City, which invests in E Ink. "It's certainly a technology that we want to involve ourselves in, at least on a test basis," he says.

On track? Hand-held book readers and railway station signs may pave the way for markets for electronic paper.

To turn their prototypes into commercial products, though, developers will have to learn to make electronic paper cheaply and in large quantities and build a manufacturing infrastructure capable of challenging existing display technologies. All that could take years, says Stewart Hough, an industry consultant with Advanced Technology Applications in Madera, California. Still, Hough says, companies such as E Ink and Gyricon have found market niches that should keep them afloat until the technology matures. "You've got to fund your habit," he says. And even skeptics expect the first bona fide electronic paper products to reach the market within a decade or so. Electronic paper is coming. The question is when will it arrive—and what will it do when it gets here?

–MARIE GRANMAR AND ADRIAN CHO

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Changes in the Sun May Sway The Tropical Monsoon

Oxygen-isotope patterns in a stalagmite from a cave in southern China indicate that the sun is one of the main drivers of tropical monsoon variations over the centuries

Scientists have long presumed that a changeable sun might influence climate. Decades before satellite observations in the 1980s showed slight fluctuations in our star's brightness, researchers were hunting for evidence of a sun-climate connection. That search has made halting progress, however. Researchers have had trouble finding both proof of such a connection and an explanation for how it might work.

Now evidence is accumulating that solar variations have altered at least one aspect of climate, the rain-laden monsoonal winds that sweep in from the sea around the tropics. And there's even a new mechanism for the observed sun-monsoon link. The latest evidence "is kind of selling me on [a sun-climate link]," says longtime doubter Gerald North of Texas A&M University in College Station. Still, he adds, "the big mystery is that the solar signal should be too small to trigger anything" in the climate system.

The latest and some of the best evidence for a sun-monsoon link comes from a rock grown in a cave in southern China. On page 854, geologist Yongjin Wang of Nanjing Normal University in China and colleagues at Nanjing and the University of Minnesota, Twin Cities, report their analysis of a meter-long stalagmite that grew during the past 9000 years in Dongge Cave. Each added layer of carbonate mineral recorded the oxygen-isotope composition of the monsoon rains that were falling on southern China at the time, dissolving carbonate rock and redepositing that mineral as a stalagmite at about 100 micrometers per year. Monsoon rains upwind of the cave lighten the oxygen isotopes of rain falling at Dongge. So, the lighter the stalagmite oxygen isotopes are, the wetter the summer monsoon was when that bit of stalagmite formed. The team dated the layers radiometrically, using uranium and thorium isotopes.

The stalagmite revealed that cycles of 558, 206, and 159 years, on average, are superimposed on a jumble of variations in monsoonal rains since the last ice age. These climate periodicities resemble those in the record of varying carbon-14 in tree rings, the authors note, cycles widely attributed to variations in solar activity. In fact, the two records have a half-dozen periodicities in common. "This matching suggests that the intensity of the summer [East Asian] monsoon is affected by solar activity," says Minnesota team member Hai Cheng. "The sun is one of the main drivers" of monsoonal climate change on centennial time scales, he says.

Millennial rains. This cross-sectioned stalagmite recorded 9000 years of rain influenced by solar variations.

CREDIT: Y. WANG *ET AL.*

That's a strong contention in a field littered with debunked claims and disappearing correlations, but the existence of a solarlike signal in monsoon climate records is getting hard to ignore. "The correlation is very strong" in the Dongge record, says North. "I find it very hard to refute." "This is probably the best monsoon record I've seen," adds paleoclimatologist Dominik Fleitmann of Stanford University in Palo Alto, California. "Even better than ours." That was a stalagmite record from southern Oman, 5000 kilometers to the west under the Indian Ocean monsoon, published in *Science* in 2003. It too showed solar signals, as have two other stalagmites from Oman, lake sediment records from East Africa another couple of thousand kilometers to the west, and a just-published second stalagmite record from Dongge. "Now we can ask the modeling community to provide a mechanism" for linking solar activity and the monsoon, Fleitmann says.

As it happens, modelers do have a new sun-monsoon mechanism to offer. Gerald Meehl of the National Center for Atmospheric Research (NCAR) in Boulder, Colorado, and colleagues published a study in the *Journal of Climate* in 2003 in which they traced the effects of a brighter sun through the climate system. In their model, increased solar irradiance amplified the heating of the relatively cloud-free subtropics, which boosted evaporation there. When winds carried the additional moisture into monsoon regions, it condensed, increasing monsoonal rains and intensifying the winds that drive the whole system.

Meehl, meteorologist Harry van Loon of Northwest Research Associates in Boulder, and meteorologist Julie M. Arblaster of NCAR have now found signs in the real atmosphere of what appears to be their model's sun-monsoon mechanism at work. Writing in the December 2004 *Journal of Atmospheric and Solar-Terrestrial Physics*, they report that the whole tropical circulation system that feeds monsoons around the world intensified and weakened over the past 50 years in step with the 11-year solar cycle. When the sun was brightest, rains were heavier not just in the Indian monsoon but in almost every region of localized tropical precipitation around the world, from the North American monsoon (in the American Southwest) to the Sahel of West Africa.

Things may be looking up in sun-climate relations, but in this field especially, looks can be deceptive. In sun-climate, "just when you think you're making progress on one front, something on another front falls apart," says solar physicist Judith Lean of the Naval Research Laboratory (NRL) in Washington, D.C. Modelers, including Meehl, have been using her estimates of the sun's brightness variations centuries before satellite observations to try to match the timing and magnitude of past climate variations.

Now Lean questions her brightness estimates. She based them on the analogy of solar brightness with the brightness distribution of sunlike stars, but that analogy is not holding up, she says. With NRL colleagues Yi-Ming Wang and Neil Sheeley, she will shortly publish revised estimates, based solely on known behavior of the sun, that are only one-quarter the size of her star-based estimates. "Until we know what the carbon-14 record is telling you, all this has some uncertainty," Lean says. "We have a lot to learn about the sun before we know what the past irradiance was."

–**Richard A. Kerr**

Random Samples

Edited by Constance Holden

Mystery Toad Blowups

It isn't easy being green, as the saying goes. That's especially true if you're a toad in Germany. Over the past week, toads have reportedly been spontaneously exploding around a small pond in an upscale Hamburg suburb. Observers report that the amphibians crawl out of the water, swell to several times normal size, and then burst, their guts shooting a meter into the air. More than 1000 carcasses have been collected so far. The area has been dubbed the "Pond of Death" and closed to the public.

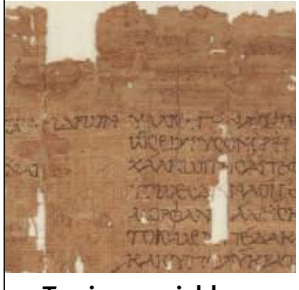
The Hamburg Conservation Alliance is organizing an investigation, but so far scientists are stumped. The top theory was that a pathogenic fungus from South America known to cause bloating had found its way into the pond via foreign horses at a nearby racetrack. But no traces of the fungus, or of any foreign bacterium or virus, have been found in the tissues of exploded toads. Nor have any toxins been found in tests of the water or in other pond organisms.

A Berlin veterinary surgeon has suggested that crows peck holes in toads to get at their livers, and the toads swell up in self-defense. But experts are skeptical. Samples of exploded toad tissue are being sent to labs, and it may take weeks to clear up the mystery. Michael Berrill, an amphibian pathologist at Trent University in Peterborough, Canada, says he "would have to see it to believe it." Although he's seen frogs bloated from bacterial infections, he's never seen an inflated toad.

Camera Unobscura

Using a technique developed to analyze satellite photos, scholars have read heretofore undecipherable fragments of ancient papyrus and have discovered snatches of lost texts by Sophocles, Lucian, and Euripides, among other treasures.

The fragments are from the famed *Oxyrhynchus Papyri*, 400,000 written artifacts that were dug up from rubbish heaps around the ruins of the eponymous Egyptian city a century ago. The collection includes official documents, private letters, lists, and literature, and it has already given scholars unparalleled insight into daily life in the ancient provincial capital. But many bits of the papyri are too badly discolored to read, says Dirk Obbink, a classicist at Oxford University in the U.K., which houses the collection.



Top image yields more from a lyric poem by Ibycus.

Obbink enlisted electrical engineer Gene Ware and colleagues at Brigham Young University in Provo, Utah, to study samples using multispectral imaging. The researchers took multiple digital photographs of each piece. Each photo captured light of a different wavelength, and by analyzing the images, the researchers could see through the grime and enhance the contrast between ink and papyrus. That increased the number of readable bits by about 20%, Obbink says. The findings will be published this summer by the Egypt Exploration Society in London.

Even though most pieces contain only a few sentences or words, says Todd Hickey, a papyrologist at the University of California, Berkeley, "you'd be surprised how much you can get out of a postcard-sized fragment."

Wind Power in Wordsworth Country

Two major environmental groups are telling Brits who would live near a proposed wind farm: Yes, in your backyard. Although known for protecting open spaces against development, London's Greenpeace U.K. and Friends of the Earth have now endorsed plans for a wind farm in the north of England. "It's not often we're supporting a particular development," says Douglas Parr, chief scientist at Greenpeace U.K. "But we need energy."

The project would dot 27 turbines across a windswept 4-kilometer stretch of countryside in the Lake District, the bucolic landscape in which the poet William Wordsworth lived and drew inspiration. The setup, near Tebay, would provide up to 80 megawatts of power, enough for at least 47,000 homes, the developers say.

Photomontage put together by wind-farm opponents. (Turbines are scaled to about projected size.)

Some conservation groups are protesting the incursion, saying that the white, 110-meter-tall turbines will spoil the scenery and hurt tourism. "It is a relatively wild landscape" that should stay free of "manmade structures," says Ken Burgess of the U.K. government's Countryside Agency. The agency was to vote this week on whether to attempt to block the development by recommending designation of the area as a national park.

The U.K. Department of Trade and Industry will decide whether to approve the plan pending a 7-week public inquiry. Such projects are a step toward reaching the government's goal of raising the proportion of electricity the country gets from renewable sources from 3% to 10% by 2010.

CREDITS (TOP TO BOTTOM): PHOTOS.COM; DAVE MULLIGAN/NVM DIGITAL.COM; THE OXYRHYNCHUS PAPYRI PROJECT, OXFORD, AND THE EGYPT EXPLORATION SOCIETY

Edited by Yudhijit Bhattacharjee

JOBS

Back in business. After 4 years in academia, computer scientist and entrepreneur Jeong Kim is returning to Lucent Technologies in Murray Hill, New Jersey, as president of Bell Labs. He succeeds James O'Shea, who's retiring after 33 years at the company.

A former nuclear submarine officer for the U.S. Navy, Kim entered the corporate limelight in 1992 when he founded Yurie Systems Inc., a high-tech communications equipment company. In 1998, he made a fortune and was absorbed into Lucent when the telecom giant acquired Yurie Systems Inc. for $1 billion. He later served as president of Lucent's Optical Network Group before joining the University of Maryland, College Park, in 2001.

Kim's "considerable experience, entrepreneurial spirit, and proven track record in both commercializing new technology and leading high-performance technical teams" make him an ideal choice for the job, says Patricia Russo, chairperson and CEO of Lucent Technologies.

Got any tips for this page? E-mail people@aaas.org

Skyward. A former high school science teacher will take over the Multi-Mirror Telescope (MMT) Observatory atop Mount Hopkins in Amado, Arizona. It will be a homecoming of sorts for Faith Vilas, 53, who helped detect Neptune's rings in 1984 while a doctoral student at the University of Arizona in nearby Tucson.

Now leader of the planetary astronomy group at NASA's Johnson Space Center in Houston, Texas, Vilas hasn't been around telescopes in years. But selection committee members say her technical and managerial expertise make her a perfect choice to head the facility, run jointly by the Smithsonian Institution and the University of Arizona.

"The MMT represents some of the finest facilities that astronomy has today," Vilas says about the combination of a 6.5-meter telescope and a collection of instruments. A Japanese mission to rendezvous with an asteroid in September will keep her busy until December.

AWARDS

• Forest ecologist Jerry Franklin, theoretical physicist Sidney Drell, and condensed matter physicist Mildred Dresselhaus are among the winners of this year's Heinz Awards. Franklin receives the prize in the environment category for his contribution to conserving America's forests; Drell in the public policy sphere for his work on reducing the danger and proliferation of nuclear weapons; and Dresselhaus in the category of technology, the economy, and employment for expanding opportunities for women in science. Each winner receives $250,000.

• Massachusetts Institute of Technology chemical engineer Robert Langer has won the $500,000 Albany Medical Center Prize in Medicine and Biomedical Research.

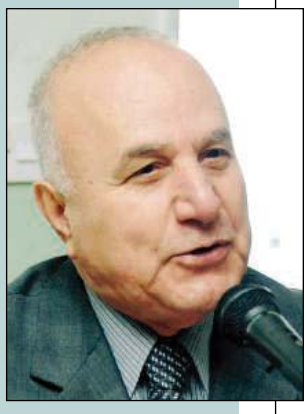
Inexpensive science. A half-million-dollar research award would not go very far in most scientific disciplines. But it can be "a lifetime of funding for a social scientist," jokes the latest winner of the Alan T. Waterman Award from the U.S. National Science Foundation.

Dalton Conley is the first sociologist ever to win the prize, which has been awarded annually since 1975 to a promising researcher age 35 or under. A professor at New York University, Conley receives the honor for his examination of how socioeconomic status gets transmitted through generations.

Besides being personally rewarding, the award should help counter the insecurity that some sociology researchers have about "not being taken seriously as scientists," says Conley. He plans to use the money, which he will receive over 3 years, to hire more graduate students and collaborate with biologists on studying the effect of genes and environment on human behavior.

POLITICS

Cause for cheer. Biologist Adnan Badran has already spent much of his career boosting science in Jordan by helping establish two universities and authoring 15 textbooks. Now, he has a chance to take it to the next level as the first scientist to lead the Jordanian government.



Badran, 70, who last month was appointed prime minister by Jordan's monarch, King Abdullah II, says his first goal will be to double the science budget from its current $100 million. In addition to combating a chronic brain drain, Badran is looking for "some big, cross-disciplinary projects that will get our scientists working together." His wish list includes solar energy, salt-resistant crops, and a controversial plan to replenish the Dead Sea by connecting it to the Red Sea. He also hopes to preside this year over the start-up of the SESAME synchrotron research facility.

"Financial times are rough here," says Mohammad Hamdan, a computer scientist at Yarmouk University in Irbid, Jordan, "but now we have someone who will do his utmost for science."

CREDITS (TOP TO BOTTOM): AP; J. PAMELA PHOTOGRAPHY; LUCENT TECHNOLOGIES/BELL LABS; EDWIN AMENTA

Q AAAs

Who's making *Science* a household name?

“ I am always thrilled to receive my personal edition of *Science*, which I consider to be one of the best scientific magazines on Earth. I read it undisturbed and absorbed at home. Thank you, *Science,* for being so informative, knowledgeable, and abreast of times, and for giving me the intellectual stimulation I crave. ”

Professor Fioretta Benedetto Mattia, AAAS member

Photo: Planet Photo Studio, Milan, Italy

Fioretta Benedetto Mattia at home in Milan, Italy

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Calling on Scientists to Fight Budget Cuts

THE FUNDING LEVELS REQUESTED BY THE Bush Administration for 2006 ("Caught in the squeeze," J. Mervis, News Focus, 11 Feb., p. 832) represent a decrease in science and technology funding across the board. This budget and its priorities do not bode well for American science and technology or for America's scientists and science students. Underfunding science and technology research and education today is short-sighted. It puts our nation's strong global standing in science and technology at risk now and in the future.

As ranking member of the U.S. House of Representative's Committee on Science (which has jurisdiction over all nondefense science research and development including the National Science Foundation), I am familiar with the realities of our country's current fiscal crisis and attempts to "remedy" that situation by cutting "lesser priorities." I assure you that some Members of Congress, including myself, are fighting to push science and technology as a priority in this and future budgets.

However, Congress cannot achieve this alone; we must have your help. Adding your voices to ours is essential in presenting a unified front in support of additional science and technology funding. In a time of necessary fiscal restraint, advocates of science must be vocal in communicating science's centrality to our nation's future. It must be clear that science is not just an academic exercise.

The current downward trend in funding can be reversed. The federal budget is not irrevocably set and can be redrawn. Researchers, students, faculty, this affects you. Write, call, e-mail, and speak on the importance of what you do for this nation's economy. Help us help you by being your own unrelenting advocates.

BART GORDON*

U.S. House of Representatives, Ford House Office Building, H2-394, Washington, DC 20515, USA.
*U.S. Representative for Tennessee's Sixth Congressional District; Ranking Member, House Committee on Science.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

Establishing Indicators for Biodiversity

IN THEIR POLICY FORUM "THE CONVENTION on Biological Diversity's 2010 target" (14 Jan., p. 212), A. Balmford *et al.* argue that "conservation scientists have a lot to learn… from economists" in regard to the establishment of indicators that are "rigorous, repeatable, widely accepted, and easily understood." By way of example, they refer to gross domestic product (GDP) and write that the "global imperative to protect biodiversity and ecosystem services must become as politically significant as economic growth…"

GDP may be a repeatable and widely accepted measure, but it is not rigorous and it is easily misunderstood. GDP measures a country's dollar market value of legal, final (nonintermediate) goods and services produced during the course of an accounting period, such as one year. That can be a problem. Consider two examples: First, people become ill on account of pollution and have to seek medical treatment; more medical services are produced and counted in GDP at their market value. GDP rises. Economies grow. But we are not better off for having been polluted in the first place. Second, the more wars we fight, the more funds governments expend in the arms market, but we cannot argue that states are better off for fighting wars. Conversely, if we become healthier and fight fewer wars, GDP falls and economies shrink.

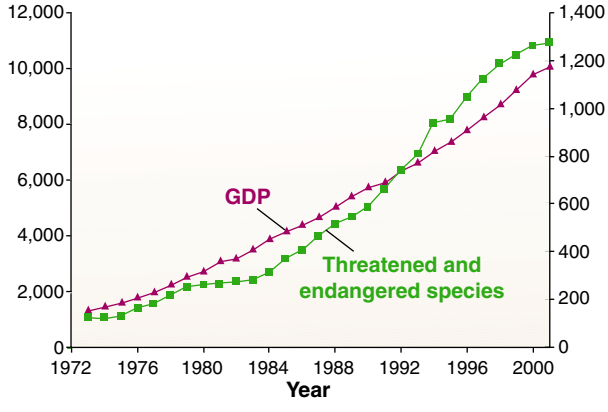
Economics can make tremendously valuable contributions to biology, but GDP and economic growth measures are not among them.

JURGEN BRAUER

College of Business Administration, Augusta State University, Augusta, GA 30904, USA, and Vice-Chair, Board of Directors, Economists for Peace and Security, Annandale on Hudson, NY 12405, USA. E-mail: jbrauer@aug.edu

IN THEIR POLICY FORUM "THE CONVENTION on Biological Diversity's 2010 target" (14 Jan., p. 212), A. Balmford *et al.* describe the need for biodiversity indicators pursuant to the Convention on Biological Diversity. They identify gross domestic product (GDP), a measure of economic growth, as a precedent-setting indicator to be emulated by scientists. We propose that scientists already possess such an indicator, namely, inverse GDP.

As indicated by rising GDP, economic growth entails increasing population times per capita consumption (*1*). Technological progress broadens the human niche (*2*); economic growth is the process of filling the broadened niche (*3*). Economic growth entails the reallocation of natural resources from the "economy of nature" and its nonhuman species to the human economy (*4*).



U.S. GDP correlates with the number of U.S. threatened and endangered species. GDP figures are in billions of dollars (www.bea.doc.gov/bea/dn1.htm). Threatened and endangered species are those listed by 31 December of the corresponding year (http://ecos.fws.gov/tess_public/).

The tight correlation ($R^2 = 0.99$; see figure) of U.S. GDP to the number of U.S. threatened and endangered species listed under the Endangered Species Act is unlikely to be a coincidence. The sectors comprising the economy are the same sectors endangering species (*5*).

Some may object, citing the "environmental Kuznets curve," the hypothesis that the environment deteriorates during early phases of economic growth, then recovers after a threshold of growth is achieved (*6*). However, environmental Kuznets curves are thought to apply to only a limited set of pollutants (*7*), not to environmental issues stemming from macroeconomic activity (*8*). Biodiversity is threatened by economic sectors in the aggregate (*5*), and certainly a higher GDP cannot resurrect an extinct species.

GDP accounting is an indicator of the size of an economy, not necessarily of human welfare (*9*), and has been overlooked as an indicator of biodiversity loss.

BRIAN CZECH,[1] DAVID L. TRAUGER,[1] JOSHUA FARLEY,[2] ROBERT COSTANZA,[2] HERMAN E. DALY,[3] CHARLES A. S. HALL,[4] REED F. NOSS,[5] LISI KRALL,[6] PAUL R. KRAUSMAN[7]
[1]Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, National Capital Region, Alexandria, VA 22314, USA. [2]Gund Institute for Ecological Economics, University of Vermont, Burlington, VT 05405–1708, USA. [3]School of Public Policy, University of Maryland, College Park, MD 20722, USA. [4]College of Environmental Science and Forestry, State University of New York, Syracuse, Syracuse, NY 13210, USA. [5]Department of Biology, University of Central Florida, Orlando, FL 32816, USA. [6]Economics Department, State University of New York, Cortland, Cortland, NY 13045, USA. [7]School of Natural Resources, University of Arizona, Tucson, AZ 85721, USA.

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Response

AS BRAUER NOTES, IT IS WIDELY ACKNOWLedged that GDP is a flawed and distorted index. However, our Policy Forum was not a panegyric for GDP, but a "call to arms" to develop appropriate indices of biological diversity that are both scientifically and statistically sound and receive as much attention in the media and the minds of the public as GDP or the Dow Jones Index. This in turn leads us to dismiss the use of GDP as an index of biodiversity loss, as proposed by Czech *et al.* Although it is discouraging that GDP has a strong correlation with rates of biodiversity loss, we do not think that this qualifies it as an index for monitoring the state of environmental degradation. On the contrary, the same flaws that undermine its utility as an index of a nation's economic well-being will only be compounded when it is used as an index of environmental stress.

Inverse GDP used as an index of environmental damage instantly creates the impression that environmental protection and economic progress act in direct opposition to each other. We believe that this is not necessarily the case; the Millennium Development Goals and the Convention on Biological Diversity 2010 (CBD2010) goals directly imply that we need to find ways to develop the global economy while also protecting the environment and the welfare of those whose health and economic well-being are most dependent on the ecosystem services supplied by the natural environment. Although slowing economic growth may be desirable in wealthy countries (as Czech *et al.* argue), we believe that in poorer parts of the world this would be not only impractical but morally unacceptable.

Plainly, the principal criticism of GDP stems from the fact that it amalgamates a variety of processes into a single figure. This will also be a problem with any single index we propose to monitor biodiversity; a possible way to reduce this criticism would be to develop a set of indicators attuned to different aspects of ecosystem health. Comparing the relationship between such indices with GDP and other indices of economic progress, at a range of geographic scales, will lead to the development and testing of environmental indices that provide important insight into the health of the planet. The regular reporting of their changing value can play a crucial role in influencing public policy.

An Amazonian fisherman displaying a catfish for sale. Inverse GDP should not be used as an index of biodiversity because in poorer parts of the world economic development consistent with preservation of biodiversity must occur.

ANDREW P. DOBSON,[1] ANDREW BALMFORD,[2] PETER R. CRANE,[3] RHYS E. GREEN,[2] GEORGINA M. MACE[4]
[1]Department of Ecology and Evolutionary Biology, Princeton University, Eno Hall, Princeton, NJ 08544, USA. [2]Conservation Biology Group, Zoology Department, Cambridge University, Downing Street, Cambridge CB2 3EJ, UK. [3]Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, UK. [4]Institute of Zoology, Zoological Society of London, Regents Park, London NW1 4RY, UK.

Memo to NASA: Finish What You Start

NASA'S UPCOMING DECISIONS ON THE FATE of the Hubble Space Telescope and other valuable scientific satellites and probes ("Confusion at the space agency," D. Kennedy, Editorial, 11 Mar., p. 1533; "NASA plans to turn off several satellites," A. Lawler, News of the Week, 11 Mar., p. 1541) illustrate a fatal flaw in NASA's reasoning and planning. The loss of data and knowledge from those spacecraft has been and will be caused by over 30 years of NASA's dedication to starting projects and not finishing them. NASA is basically a propulsion agency: great on launching spacecraft, poor on following up on their advantages. Usually, there is not enough money to analyze all the data that are collected, the spacecraft may not be allowed to complete their missions, and using working spacecraft is given lower priority than launching new ones. As one who was in on the beginning and the continuation of the Landsat program, I feel I can give voice to my concerns.

If I could give one piece of advice to the new Administrator of NASA, it would be: "If you're going to start a job, finish it!"

CHARLES J. ROBINOVE
2635 Crestwood Drive, Monument, CO 80132, USA.

The End of a Chilean Institute

THE CHILEAN GOVERNMENT AND THE WORLD Bank launched the Millennium Initiative in 1999, to promote the development of "world-class" scientific centers in Chile. As a result, three Millennium Institutes have been created since 2000 for 10-year terms, subject to periodic evaluations.

The Chilean government has recently decided against the renewal of the Millennium Institute of Cellular Biology and Biotechnology (CBB). This decision is particularly surprising, because the CBB has been widely recognized internationally as one of the most successful and productive centers of excellence in Chile and as a successful example of scientific initiatives in developing countries. The Institute has published over 200 scientific papers in journals indexed by ISI, generated 25 Ph.D. graduates, and carried out an outstanding program on science education targeting the high school system in Chile.

Some of us, as members of the Institute's Advisory Panel, have given courses, performed collaborations, or attended scientific meetings at the Institute. We have been impressed by the excellent level of science at the CBB.

CREDIT: A. DOBSON

It seems a paradox that the Chilean government has terminated support to the CBB, even though the Chilean Congress has already approved the funds for the Institute to continue. The interruption of CBB activities would be a distressing sign to the international scientific community and would cast doubt on the stability of long-term scientific cooperation with Chile, with negative consequences and impact for a country that has made a significant effort to promote science and international scientific cooperation.

LUIS BARBEITO,[1] JEROLD CHUN,[2] LESTER I. BINDER,[3] VIVALDO MOURA NETO,[4] GEORGE PERRY,[5] CLAUDIO SCAZZOCHIO,[6] GALILEO VIOLINI[7]

[1]Instituto de Investigaciones Clemente Estable, Avenida Italia 3318, Montevideo 11600, Uruguay. [2]The Helen L. Dorris Child and Adolescent Neuropsychiatric Disorder Institute, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA. [3]Alzheimer's Disease Center, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA. [4]Universidade Federal do Rio de Janeiro, Prédio de Reitoria 2, Rio de Janeiro, RJ 68536, Brazil. [5]Institute of Pathology, Case Western Reserve University, 2085 Adelbert Road, Cleveland, OH 44106, USA. [6]Département de Microbiologie, Faculté des Sciences, Université Paris XI, Cedex, Orsay, Paris 91405, France. [7]Departament of Physics, Universita Della Calabria, Ponte P. Bucci - cubo 31C, Rende, Cosenza I-87036, Italy.

TECHNICAL COMMENT ABSTRACTS

Comment on "Energetics of Hydrogen Bond Network Rearrangements in Liquid Water"

A. Nilsson, Ph. Wernet, D. Nordlund, U. Bergmann, M. Cavalleri, M. Odelius, H. Ogasawara, L.-Å. Näslund, T. K. Hirsch, L. Ojamäe, P. Glatzel, L. G. M. Pettersson

Smith *et al.* (Reports, 29 October 2004, p. 851) reported a temperature-dependent x-ray absorption study on liquid water. We argue that both the measurement and the data analysis have serious shortcomings. The spectra are affected by experimental saturation effects, and the analysis suffers from incorrect assumptions for x-ray absorption spectroscopy.

Full text at www.sciencemag.org/cgi/content/full/308/5723/793a

Response to Comment on "Energetics of Hydrogen Bond Network Rearrangements in Liquid Water"

J. D. Smith, C. D. Cappa, B. M. Messer, R. C. Cohen, R. J. Saykally

We demonstrate that the spectra reported in our study are free from artifacts induced by saturation effects. Furthermore, our analysis of the energetics of hydrogen-bond rearrangement is in perfect agreement with temperature-dependent populations previously reported by Wernet *et al.*

Full text at www.sciencemag.org/cgi/content/full/308/5723/793b

ARCHAEOLOGY

Stories from a Neolithic Site

Steven Mithen

The Goddess and the Bull
Çatalhöyük: An Archaeological Journey to the Dawn of Civilization
by Michael Balter

Free Press, New York, 2005. 424 pp. $27, C$39, £18.99. ISBN 0-7432-4360-9.

Between about 7500 and 6000 BCE, several thousand people lived within a dense cluster of mud-brick buildings surrounded by marshes in what is now central Turkey. Their houses have long since collapsed to leave a low mound known as Çatalhöyük on the now relatively arid Konya Plain. Each summer, this site becomes home to well over 100 archaeologists, who are conducting one of the most ambitious archaeological projects in the world today.

The site was discovered in 1958 by James Mellaart, whose excavations in the early 1960s revealed wall paintings and figurines—including depictions of bulls, leopards, vultures, and what he interpreted as "Mother Goddesses"—that astonished the academic world. In 1993, Ian Hodder, now an archaeologist at Stanford University, began a new campaign of excavations and research at the location. That campaign, intended to last for 25 years, has become as much a cause célèbre as the site itself. Several specialist publications have already provided archaeologists with new insights into Çatalhöyük, some of which have reached a wider academic readership. In *The Goddess and the Bull*, Michael Balter—a journalist who has reported on Çatalhöyük for *Science*—seeks to bring the site to a far wider readership by writing a history of its discovery and describing the ongoing excavations and methods of interpretation.

To do so, Balter had to resolve a problem that faces anyone writing an extended account of prehistory for nonspecialists: how to maintain the readers' interest in tales of lifeless mud walls, stone tools, wall paintings, animal bones, and human skeletons. Without personalized accounts of individual experiences, the descriptions of even spectacular art and exotic burials can become tedious. Readers need to be drawn into the scientific details and academic debates through stories of people with whom they can identify and, ideally, empathize. Balter provides such characters by making the Çatalhöyük archaeologists as much the subject of his book as the site itself. By weaving their personal journeys to Çatalhöyük into his account of the site's archaeology and the development of the Neolithic, he has produced a compelling read, one that achieves the double act of educating and entertaining.

Balter successfully captures the atmosphere and spirit of a large excavation: the coming together of people from around the world to live and work together; the multiple demands on the director not only to run the excavation but to raise funds, build a team, secure permits, engage with local politicians, produce results that satisfy both his sponsors and his scientists, and so forth; the periods of strain as the end of the digging season approaches; the childlike elation felt by even the most experienced archaeologist when exciting finds are made; the evolving relations among members of the field team; the sheer academic challenge of trying to reconstruct the past from the discarded scraps of prehistoric rubbish that have survived the millennia. Every dig can provide a fascinating story of this type, but that at Çatalhöyük is unquestionably one of the best owing to the remarkable site and the equally remarkable people involved in the project.

Subject to interpretation. Most clay figurines found at Çatalhöyük are very schematic—is this a cow or a dog? Debates continue as to whether they were religious objects, game pieces, or children's toys.

The book's most intriguing figure is Ian Hodder, an ex-Cambridge archaeologist who spent much of the 1980s advocating what he termed "post-processual" archaeology (which replaced the quest for objective knowledge about the past with subjective interpretation of archaeological remains). Hodder's approach questioned the adoption of a scientific methodology that had been undertaken by "processual archaeology." (That paradigm, particularly championed by the American archaeologist Lewis Binford, held that when analyzed properly, archaeological materials revealed the lives of their makers and was initially presented as a means to discover the "laws of cultural change.") We learn about Hodder's academic career, how he devised the Çatalhöyük project to put his post-processual methodology into action, and some aspects of his personal life—as we do about many of the archaeologists who have become engaged in the project. These fascinating details serve to enhance the readers' understanding not only of how prehistoric sites are studied but also of the exceptional levels of commitment required to build a career in archaeology.

To tell the story of Çatalhöyük in this manner, Balter had to become the classic participant-observer of social anthropology. He spent a considerable time at the dig itself, where he didn't just record but also joined in the parties. He engaged in challenging academic debates with Hodder and his team, and he has put forward his own views as to how the Neolithic developed. Balter evidently has a considerable respect for all of the archaeologists he describes and affection for many. But herein lies my concern: having become an insider to write the book, has he then detached himself sufficiently to provide an account that amounts to more than a eulogy of Hodder and his project?

At times this is how the book reads, but Balter is in fact deploying his journalistic skills with considerable subtlety. Take, for instance, the way he treats one of the greatest fallacies of post-processual archaeology—that it introduced the idea that interpretation of archaeological remains begins "at the trowel's edge," to quote Hodder. Balter explains that this means an excavator within a trench must adopt a thoughtful and self-critical approach to what he or she is doing down there. Well, of course, how could it be otherwise? This has been known and practiced by excavators since the discipline began. The contrast does not lie between processual and post-processual

The reviewer is at the School of Human and Environmental Sciences, University of Reading, Shinfield Road, Whiteknights, Reading, RG6 6AB, UK. E-mail: s.j.mithen@reading.ac.uk

CREDIT: ÇATALHÖYÜK RESEARCH PROJECT, CAMBRIDGE

archaeology, as Hodder has repeatedly claimed, but simply between good and bad field technique. Balter knows enough about archaeology that he could have easily taken Hodder to task as to whether his field methodology is in reality any different from that which has been regularly practiced within the discipline long before the rhetoric of post-processual archaeology was devised. Instead, Balter voices this view through the thoughts of an archaeologist (one on whom Hodder is utterly reliant for the success of the excavation) digging by herself early one morning. By so doing, Balter avoids imposing his own judgment while still exposing an underlying sense of unease that not all is quite as right about the project as might initially appear.

It is still too early to evaluate the success of Hodder's Çatalhöyük project. Indeed, Balter has had to work hard to extract new information about the site and to make that which has been discovered sound exciting (because the new analyses show that Çatalhöyük has more in common with other Neolithic sites than Mellaart had believed). The site is nonetheless fascinating, but most readers will be just as intrigued by the archaeologists themselves. They are an extraordinarily committed team—or rather teams, as there has already been a considerable turnover of staff. Many are passionate about the site, which will form the basis for their own careers and will certainly provide life-long friendships and memories. In spite of the post-processual rhetoric that surrounds the project, they are engaged in some fantastic science at a truly remarkable prehistoric site. With *The Goddess and the Bull*, Balter serves them well by offering both the story of their site and their own stories to what will surely be a very wide readership.

10.1126/science.1110796

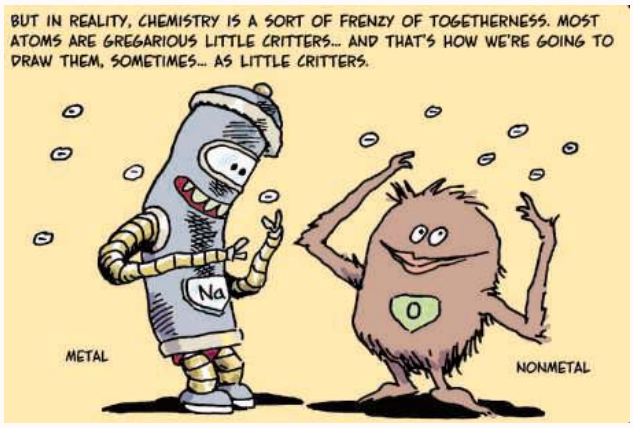
NOTA BENE: CHEMISTRY

Fun with Atoms and Molecules

The Cartoon Guide to Chemistry
by Larry Gonick and Craig Criddle

HarperResource (HarperCollins), New York, 2005. 255 pp. $16.95, C$23.95. ISBN 0-06-093677-0.

For many people, introductory chemistry is a tough sell. Part of the problem is that the first courses tend to approach the subject from two opposite directions, more or less at the same time. On the one hand, chemists study phenomena that we see, feel, and smell: Why is fire bright and hot? Where does the wood go after it burns? What causes the odor in vinegar or rotten eggs? On the other hand, several centuries of careful experiments have established a great edifice of theory, and chemical reactivity can now be described from the bottom up—starting with protons and electrons and building molecules that we can just barely conceptualize, let alone see with our eyes. The beauty of modern chemistry is that these two modes of thought merge seamlessly into a microscopic description of a macroscopic world. The challenge is that, until students digest the basics, the two modes can compete with each other and create a hopeless muddle: countless formulas to memorize and equations to balance, with little grasp of how it all relates to the bubbling solution in the beaker.



With *The Cartoon Guide to Chemistry*, Larry Gonick valiantly tries to save the day. Having previously tackled physics and genetics, the talented comic artist has now taken on what chemists proudly call the central science. To help in his endeavor, he recruited co-author Craig Criddle, a professor of environmental engineering and aquatic chemistry at Stanford University. The book's format uses cartoon panels interspersed with brief, explanatory blocks of text. Though often funny, the book is no joke. The authors have made a determined effort to present the fundamental principles of chemistry in an accessible and enjoyable fashion. For the most part, the result seems destined to succeed.

Gonick gets off to a strong start with a whirlwind history of chemistry, from the Stone Age to Mendeleev's periodic table. This includes a great sequence in which an increasingly distraught Aristotle must watch from the margins as his cherished doctrines collapse, one by one, in the face of experiments. Next comes an introduction to protons and electrons, the elements, and various trends revealed by the periodic table (e.g., atomic size, ionization energy, and electron affinity). The third chapter, "Togetherness" (on chemical bonding), brings out the real benefit of the cartoon approach. Metallic elements become tall, cylindrical robots, with electron acceptors portrayed as small, furry monsters. Through many temperamental interactions, these creatures showcase the principles of ionic and covalent electron exchange. The panels breathe life into the rules for building molecules, and they make the microscopic world look vivid and full of fun.

Unfortunately, in the middle chapters the book loses some of its momentum. In a jarring leap back to the macroscopic world, Gonick illustrates reactivity by stranding some people on a desert island and having them make clay pots, gunpowder, and various other useful materials. He clearly means for this section to be of interesting practical relevance, but the reactions in question are rather complicated. The enticing molecular choreography sketched earlier is gone—replaced by the standard, sterile element symbols in balanced equations. Readers will find little justification for why aluminosilicate clay hardens or why potassium nitrate explodes with carbon, other than that the letters on the left rearrange to letters on the right. In addition, the periodic table is now conspicuously absent, when it is clearly needed to help make sense of the frenzy.

There are a few clever ideas in the subsequent chapters on solutions and kinetics, but the book really picks up again when it reaches acids and bases. Gonick offers a wonderful scheme to explain the conjugate concept: bases are fuzzy-headed people who gobble up protons, which show up in their heads as plus signs. Toward the book's end, the robots and monsters return to demonstrate electrochemistry. Here, as in the early chapters, the behavior of the characters highlights the underlying principles with considerable humor and clarity.

On the whole, *The Cartoon Guide to Chemistry* offers a good and informative read. Trained chemists will enjoy leafing through it, and students will certainly find solace in it when they get bogged down by their primary introductory textbook. Best of all, unlike most texts that cover so much technical ground, the book is priced for the mass market. For the public, it stands to open a big window into the small world of atoms and molecules.

–JAKE YESTON

10.1126/science.1112862

CREDIT: LARRY GONICK

Pleistocene Park: Return of the Mammoth's Ecosystem

Sergey A. Zimov

During the last ice age, the world's most extensive ecosystem stretched from France across the Bering Strait to Canada and from the arctic islands to northern China. It was at the very end of a more than million-year epoch, the Pleistocene, during which colossal ice sheets repeatedly advanced and retreated, plowing up much of northern Europe and America. At the same time, from a geological perspective, northeastern Siberia remained relatively unscathed. There, vast dust-covered plains and valleys dominated the landscape. Mammoths, woolly rhinoceroses, bison, horses, reindeer, musk oxen, elk, moose, saiga, and yaks grazed on grasslands under the predatory gaze of cave lions and wolves.

This yearlong essay series celebrates 125 years of *Science* by inviting researchers from around the world to provide a regional view of the scientific enterprise. Series Editor, Ivan Amato

The ground, as in Siberia today, froze, contracted, and cracked each winter. In spring, water penetrated and froze in deep, narrow cracks, creating networks of ice wedges. Over time, because of the slow accumulation of dust, river silt, and ice, the northern lowlands of Siberia became covered with a thick sedimentary mantle of frozen loess. These frozen sediments are filled with rootlets of grasses, microbes, and animal bones, all of which have enabled scientists to chronicle the rise and fall of the region's Pleistocene ecosystem.

About 10,000 years ago, at the beginning of the Holocene epoch, this vast system, which I refer to as the mammoth tundra-steppe, disappeared completely. In northern Siberia, mossy tundra and forest tundra replaced the mammoth ecosystem. The only herbivores to survive were reindeer that grazed on lichens and moose that fed on willows. The mammoths and their large animal companions, which had survived even the worst conditions the ice age could muster, disappeared during the Holocene warming.

It actually might not have been the climatic changes that killed off these great animals and their ecosystem, however. More consequential, perhaps, were shifts in ecological dynamics wrought by people who relied on increasingly efficient hunting practices, which decimated the very populations of grazing animals that maintained the tundra steppe. To test this possibility, my colleagues and I for the past decade have been working to reconstitute the mammoth ecosystem in one modest parcel of the northern Siberian region of Yakutia. We call our project Pleistocene Park. The primary scientific goal is to determine more precisely the role that Pleistocene animals played in maintaining their own ecosystem. However, we also suspect that by learning how to preserve and extend Pleistocene-like grasslands in the northern latitudes, we could subsequently develop means for mitigating both the progress and effects of global warming. The amount of carbon now sequestered in soils of the former mammoth ecosystem, and that could end up as greenhouse gases if released into the atmosphere by rising global temperatures, surpasses the total carbon content of all of the planet's rain forests.

The Vanishing of the Herbivores

Grassland ecosystems are evolutionarily the youngest of ecosystems. These ecosystems have the highest rates of biogeochemical cycling. Grasses use water resources more rapidly than their less productive competitors, such as cactuses and trees, rather than spending energy for making thorns and toxins to ward off enemies. When their numbers reach a level that can be sustained by the landscape, herbivores eat and trample all the grassland

Sergey A. Zimov
Russia

Sergey A. Zimov, director of the Northeast Science Station in Cherskii in the Republic of Sakha (Yakutia), received his academic training in geophysics at the Far East State University in Vladivostok, Russia. He subsequently did fieldwork in northern Siberia for the Pacific Institute for Geography, part of the Far East Branch of the Russian Academy of Sciences. In 1980, he organized the science station that he now directs. Research at the center includes studies of global carbon and methane budgets and animal extinctions that occurred in Siberia when the Pleistocene epoch gave way to the ongoing Holocene about 10,000 years ago. In 1989, Zimov initiated a long-term project known as "Pleistocene Park," which he now is pursuing with a number of partners. The goal of the project is to reconstitute the long-gone ecosystem of the Pleistocene epoch that supported vast populations of large animals including mammoths, horses, reindeer, bison, wolves, and other large predators. If the effort succeeds in the park, Zimov and his co-workers would like to see the ecosystem restored over much larger areas in an effort to stave off what otherwise could be a massive release of carbon that now is sequestered in the permafrost but that could be released into the atmosphere as global temperatures rise. His hunting of mammoth remains in the tundra and his bold vision of controlling and restoring ecosystems have earned him coverage in books, documentaries, and other media.

All essays appearing in this series can be found online at www.sciencemag.org/sciext/globalvoices/

CREDIT: DENISE BERJAK

vegetation produced during the rainy season and return nutrients to the soil through their manure. On different continents, at different latitudes, grassland ecosystems have been, and are now, composed of different species, but they share a similar set of functional types or guilds. These include grasses, elephants, horses, rodents, dung-beetles, large cats, vultures, and so on. The greater the diversity within and among these functional types, the more active the biological cycles and the more successful and extensive the ecosystem can become.

In the Pleistocene, grassland ecosystems occupied about half of the world's land mass. *Homo* species emerged in these pasture ecosystems, where they left tools, weapons, cave paintings, and other signs of their presence. Starting with unpretentious ambitions to survive in a hostile environment, *Homo* ended up assuming the powerful role of ecosystem terminator. The mammoth ecosystem was the first large-scale victim, but the global destruction of grasslands only accelerated in the Holocene when people invented agriculture and began raising cattle.

Twenty years ago, scientists explained the disappearance of numerous animals in the northern grasslands very simply—the arid steppe climate changed into a humid one, and when the steppe vanished so did the steppe's animals. In short, the moist Holocene climate was a catastrophe for them. In the last few years, however, a growing accumulation of radiocarbon dates of animal remains has been suggesting a different story. It appears now that mammoths survived the Pleistocene-Holocene shift. For the first 7000 years of the Holocene, they persisted on Wrangell Island in the Arctic Ocean. Bison, horses, and musk oxen also lived in the north of Siberia in the Holocene. Horses and musk oxen lived there even up to historical times.

In Alaska, bison survived throughout the entire Holocene. They disappeared only in the historical period at the hands of human hunters. Alaskan native elders still tell stories that chronicle the taste of bison meat. Another indication that climate change has had little to do with the survival of bison is that in the past century, bison were brought back to Alaska, and they have been breeding there successfully. What's more, when musk oxen were reintroduced from the coldest, driest islands of the Canadian Arctic to Alaska in the 20th century, they immediately began to breed actively, even though the climate in Alaska was warmer and wetter. The same thing happened wherever musk oxen were reintroduced in Siberia. Even in the west Norwegian climate, musk oxen have prospered.

The recent history of horses bolsters the case against climate change as the factor that destroyed the mammoth ecosystem and its diversity of large animals. In the Republic of Yakutia in northern Siberia, the biomass of horses is greater than that of reindeer. Although horses are classified as domesticated animals, in practice most of them are wild, living without any aid from people. Evidently, they are suited to the present climate.

Yet, these great herbivores disappeared by the millions from northern Siberia and elsewhere. As has happened elsewhere and at other times, their vanishing coincides with the introduction by humans of new hunting technology. In Australia, 46,000 years ago, when people first arrived, 23 animal species vanished, all but one heavier than 45 kg (about 100 pounds). In America, 12,000 years ago, hunters began using small, sharp lances and arrowheads. After that, 70% of the large animal species vanished. By the time people started recording their own history, bison, aurochs, dziggetai (koulan), wild horses, saiga, and many other herbivores had already been exterminated from the steppes and prairies.

CREDIT: S. ZIMOV

Horse sense. Grazing on a snow-covered tundra meadow in northern Siberia, rugged Yakutian horses like these could help reduce the effects of global warming by stabilizing vast expanses of grassland.

Out to Pasture

Just as the great herbivore herds disappeared at the end of the Pleistocene, so did the northern grasslands that nurtured them. One possible explanation for this is simply that the cold, arid climate of the steppes changed into a humid one, turning the steppes into mossy tundra. However, the Holocene climate shift was not unique. Similar shifts occurred in previous interglacial periods, yet these did not cause catastrophic landscape reconstructions.

During the last glacial, when mammoths still roamed on the steppes that covered Europe, the annual precipitation there was 200 to 250 mm, and January temperatures were in the range of –25° to 35°C. Such climate conditions are similar to those of present-day northeastern Siberia. By many criteria, the present climate there is not humid, but rather is characteristic of an arid steppe. According to all weather stations of northeast Siberia, the annual radiation input is about twice what is necessary to evaporate the annual precipitation. This only adds to the mystery of why Siberia is no longer dominated by a grassy, steppe landscape.

The physiological traits associated with Holocene vegetation partially explain the vegetation changes that coincided with loss of the Pleistocene megafauna. Plant transpiration accounts for most of the water loss from landscapes, and high transpiration rates are associated with more productive plants. Rates of water loss must therefore have been high in the north when productive Pleistocene meadow and steppe vegetation prevailed. As a result, vast amounts of water were sucked up from the ground, resulting in dry conditions, while the plants themselves sequestered nutrients to drive their own productivity.

Holocene vegetation, in contrast, is dominated by unproductive moss and shrubs. This type of vegetation does not transpire enough moisture to dry out the soil. Moss does not even have roots. This leads to wet conditions conducive to the growth of mosses, which account for a substantial proportion of the northern Siberian biomass. Water-saturated soils inhibit decomposition of biomass and therefore the availability of nutrients to support plant growth. What's more, mosses insulate the ground efficiently—a 20-cm layer of moss prevents the underlying frozen soil from thawing. This also has the effect of sequestering nutrients and preventing their cycling through the ecosystem. All of these factors indicate that moss communities, once they are in place, create and sustain their own environment and do not depend so much on particular climate conditions.

They are quite vulnerable to physical disturbance, however, and this is where their ecological connection to herbivores comes in.

The Future of the Past

When mosses are destroyed on loess soils, the site becomes overgrown with grasses within 1 to 2 years. The grasses then dry out the soil through their high transpiration rates, creating a steppe-like ecosystem. But when herbivore populations are low, grass productivity begins to decrease within a few years, because grass litter accumulates on the soil surface, shading and insulating the soil. In turn, soil fertility declines. As a result, shrubs and mosses,

which have lower nutrient requirements than grasses, ultimately become dominant.

In the mammoth ecosystem, the collective behavior of millions of competitive herbivores maintained the grasslands. In the winter, the animals ate the grasses that grew the previous summer. All the while they fueled plant productivity by fertilizing the soil with their manure, and they trampled down moss and shrubs, preventing these plants from gaining a foothold. It is my contention that the northern grasslands would have remained viable in the Holocene had the great herds of Pleistocene animals remained in place to maintain the landscape.

In the southern steppes, the situation is different. There, the warmer soil allows for more rapid decomposition of plant litter even in the absence of herbivores. In the north today, the soil is too cold to foster such decomposition, which means that the steppe ecosystem can be stable there only with the help of herbivores that decompose organic matter in their stomachs and that disturb mosses. Today's African savannas, in which trees and shrubs have supplanted grasses in much the same way that mossy tundra has supplanted grasses in Siberia, demonstrate this principle. These savannas would disappear without large herbivores, which are present there in large numbers. The large numbers of animals on African savannas amaze many people. However, similar animal densities exist in northern and middle latitudes. For example, at Elk Island National Park in Canada, about 60 bison browse on each square kilometer of grassland. The animal is much bigger than the gnus and zebra of Africa. Forests in the park are preserved only by strongly controlling the number of animals.

This is why I believe that the changing climate of the Holocene would have had little bearing on the survival of the mammoth ecosystem. In some places, such as sandy and stony ground, trees and shrubs would have appeared. And that might have caused changes in the relative proportions of horses and moose. But overall, if climate were the only controlling factor, the total pasture productivity and the number of herbivores should have increased in the Holocene. Support for this view comes from the climate history that is chronicled in the Greenland ice sheet. It shows a sharp warming and dramatic increase of precipitation ~14,700 years ago, leading to conditions that resemble the present climate. Even so, in the north of Siberia, mammoth populations soared at this time.

This view means that the present Holocene climate of northern Siberia, particularly near the present tree line, is likely just now to be optimal for the mammoth ecosystem. If we accept the argument that the pasture landscapes were destroyed because herbivore populations were decimated by human hunting, then it stands to reason that those landscapes can be reconstituted by the judicious return of appropriate herbivore communities.

In northern Siberia, mainly in the Republic of Yakutia, plains that once were covered by tens of meters of mammoth steppe soils now occupy a million square kilometers. The climate of the territory is near optimal for northern grassland ecosystems. Thus, in principle, the ancient mammoth ecosystem could be restored there.

Pleistocene Park. This territory in the Republic of Yakutia is roughly an even split of meadow, larch forest, and willow shrubland. This Siberian region could become the venue for a reconstituted ecosystem that vanished 10,000 years ago.

In Yakutia, we are trying to do just that. The government has adopted a program to restore the republic's former biodiversity. One thrust of this effort has been through the nonprofit organization of Pleistocene Park—of which I am a founding member—on 160 km^2 of Kolyma lowland. One-third of the territory is meadow, one-third is forest, and one-third is willow shrubland. Today, many of the animals of the mammoth ecosystem and grasses remain in northern Yakutia.

Reindeer, moose, Yakutian horses, recently reintroduced musk oxen, hares, marmots, and ground squirrels forage for vegetation, and predators, including wolves, bears, lynxes, wolverines, foxes, polar foxes, and sables, prey on the herbivores. However, strong hunting pressure has kept the overall number of animals low. Therefore, their influence on vegetation is small. The first step for Pleistocene Park, which we are just now initiating, is to gather the surviving megafauna of the mammoth ecosystem (initially without predators) within the part of the parkland that is rich in grassland. The second step will be to increase the herbivore density sufficiently to influence the vegetation and soil. As animal densities increase, the fenced boundary will be expanded.

The most important phase of the program will be the reintroduction of bison from Canada and subsequently, when the herbivores are sufficiently abundant, the acclimatization of Siberian tigers. In many regions of the Amur River basin, where this formidable predator survives, January temperature is as low as –25° to –30°C. The tigers' survival there is limited more by poaching and herbivore density than by climate. Scientifically, Pleistocene Park is important because it directly tests the role of large herbivores in creating and maintaining grassland ecosystems, something that can only be surmised but not proven from the paleorecord.

There is more than just scientific discovery at stake here. Northern Siberia will influence the character of global climate change. If greenhouse gas–induced warming continues, the permafrost will melt. At present, the frozen soils lock up a vast store of organic carbon. With an average carbon content of 2.5%, the soil of the mammoth ecosystem harbors about 500 gigatons of carbon, 2.5 times that of all rainforests combined. Moreover, this carbon is the relatively labile product of plant roots that were incorporated from productive steppe vegetation during the Pleistocene. As soon as the ice melts and the soil thaws, microbes will begin converting this long-sequestered soil carbon into carbon dioxide under aerobic conditions or into methane under anaerobic conditions. The release of these gases will only exacerbate and accelerate the greenhouse effect.

Preventing this scenario from happening could be facilitated by restoring Pleistocene-like conditions in which grasses and their root systems stabilize the soil. The albedo—or ability to reflect incoming sunlight skyward—of such ecosystems is high, so warming from solar radiation also is reduced. And with lots of herbivores present, much of the wintertime snow would be trampled, exposing the ground to colder temperatures that prevent ice from melting. All of this suggests that reconstructed grassland ecosystems, such as the ones we are working on in Pleistocene Park, could prevent permafrost from thawing and thereby mitigate some negative consequences of climate warming.

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The author is at Northeast Scientific Station, Pacific Institute for Geography (Far East Branch), Russian Academy of Sciences, Post Office Box 18, Cherskii, Republic of Sakha 678830, Russia. We thank G. Zimova, F. S. Chapin, and M. Chapin for their helpful comments.

CREDIT: S. ZIMOV

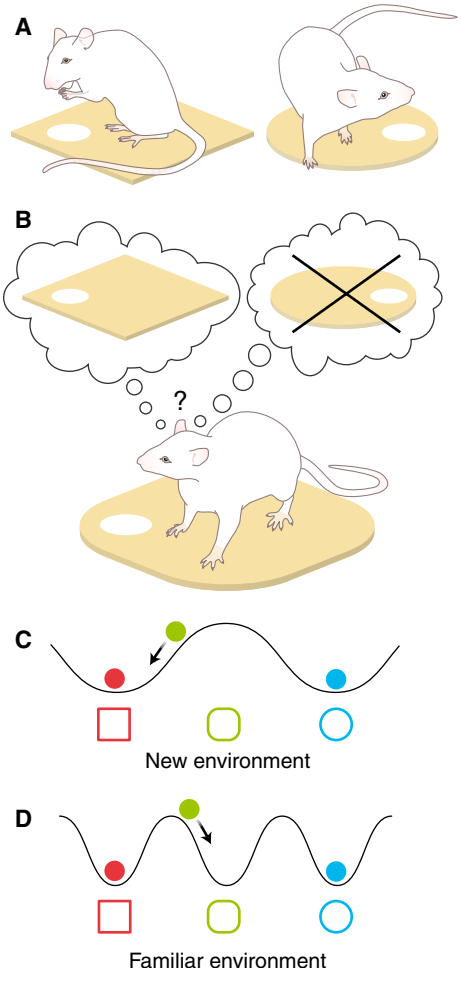
NEUROSCIENCE

Attractors in Memory

Bruno Poucet and Etienne Save

One of the most challenging questions facing contemporary neuroscience is how the brain encodes the memories of an individual's experiences. Within this broad question, the issue of how the brain makes a distinction between separate yet similar episodes is crucial. By recording the electrical discharges of neurons in the brains of animals performing different behavioral tasks, it is possible to decipher some of the rules governing this process. On page 873 of this issue, Wills *et al.* (*1*) suggest that memories are like attractor states in which all neurons abruptly and simultaneously change their electrical discharges in relation to the current experiences of the rats under study. To demonstrate this, Wills *et al.* recorded the firing activity of hippocampal place cells in the brains of freely moving rats exposed to a square or circular environment (see the figure).

The authors observed the neural activity of hippocampal place cells by recording spike activity from single hippocampal pyramidal neurons and simultaneously tracking the location of the rat in the environment. Each place cell discharges only when the animal is in a cell-specific stable region called its "place field." Place fields occur with about equal density over the entire surface of the environment, hence their ensemble firing can be decoded to determine the animal's location in space (*2*). Although place fields are stable across days and weeks in constant surroundings (*3*), they undergo great variation if large changes are made in the environment. Thus, changing the shape of the environment—for example, from a circle to a square—causes major modifications in the activity of all place cells (see the figure). Some place cells have fields in only one of the two environments and are silent in the other, whereas the fields of cells active in both environments are quite different in shape or location. Such changes, known as remappings, occur most reliably after modifying the shape of the environment, but also appear after more subtle changes (*4*, *5*). The remapping phenomenon suggests that the hippocampus learns and holds distinct maps for distinct contexts, with each specific map being reactivated as the rat commutes between the different contexts (*6*). Thus, place cells signal both the rat's current environment and its location within that environment.



Changing shapes. Rats exposed to a square or circular environment evoke distinct representations in "place" neurons of the hippocampus (*1*). (**A**) The place fields (white ellipses) are different for the circular and square environments. (**B**) When the rat is exposed to an intermediate shape (such as an octagon), all place cells simultaneously adopt one of the two learned activity patterns (the square in the example). (**C**) This observation reveals that the activity states of place cells are under the control of attractor-like mechanisms. (**D**) With experience, new attractors may develop so that intermediate shapes are represented.

The authors are in the Laboratory of Neurobiology and Cognition, CNRS–Université Aix-Marseille, Centre Saint-Charles, 13331 Marseille Cedex 3, France. E-mail: bpoucet@up.univ-mrs.fr

Given that remapping reflects the learning process of a new environment, it is of interest to investigate its time course. Remapping may be very rapid, taking place in a matter of minutes when the rat moves freely from a familiar environment to a new one (*2*). In contrast, a slower variable rat-specific time course is observed when the rat is brought from a familiar environment (such as a square) to a new environment (such as a circle) (*7*). At first, the place fields are equivalent in both environments. With additional exposures to the circle, however, the fields of a progressively greater fraction of cells become distinct. Not only does this map differentiation occur at different rates in different rats, it also occurs at different rates for cells within a given rat. Gradual shifts in the place fields may be seen for individual place cells. Ultimately, however, discrimination of the square and circle appears to go to completion so that the fields of all cells become distinct.

What is the neural correlate of this discrimination once it is established? Wills *et al.* tackled this issue by using a series of "morph" boxes. Each morph box was made of a number of juxtaposed plastic elements that could be arranged in a variety of configurations. The overall geometry of the box could be varied from a circle to a square through four intermediate octagonal shapes, from more circular-like to more square-like. Rats were first extensively exposed to a wooden circle and to a square made of morph material. This ensured the rapid occurrence of remapping, which persisted when rats were exposed to circular and square boxes both made of morph material. Then Wills *et al.* exposed rats to a series of morph boxes in a pseudo-random order across successive recording sessions.

What became of the place fields when rats were exposed to morph boxes of intermediate shape? Wills *et al.* found that most cells adopted either the circle-like or square-like pattern in the morph boxes and almost never exhibited other patterns. The switch from one activity pattern to the other was abrupt, and the switch point was for the morph box whose shape was approximately at the midpoint between a circle and a square (see the figure). Not only was the switch point similar for all rats, it was also the same for all simultaneously recorded cells within a given rat. Because only the geometry of the recording box varied across successive exposures, such effects were unambiguously caused by changes in environmental shape. Interestingly, the same abrupt changes in place fields were seen on

a second series of exposures to the morph boxes, although in some rats the shift point could vary from that displayed during the initial presentation. Moreover, the firing patterns within a session were established very rapidly, usually within the first 30 seconds.

What do these results suggest about the neural processes of memory in the brain? First, all place cells were seen to switch abruptly between two distinct states across successive sessions, which seems to indicate the operation of attractors. Such attractors induce the hippocampal system to adopt one of the two possible activity states triggered by the two well-learned box shapes. This major property of hippocampal representation is akin to pattern separation, which allows slightly different inputs to result in distinct output representations. Such a process reduces interference between similar experiences and complements another process necessary for memory retrieval; pattern completion, in which an autoassociative network recalls stored patterns based on incomplete information (*8*). Second, the results reveal that the hippocampal place cell system can build categories within which the representation of each intermediate box shape is nested. Although these properties of pattern separation and categorization are useful for initial encoding of new experiences within familiar contexts, they must also be accompanied by additional mechanisms that allow incremental storage of new contexts. Thus, with repeated exposure, the geometry of a new environment could ultimately lead to a new activity state that may permit the encoding of new memories (*7*).

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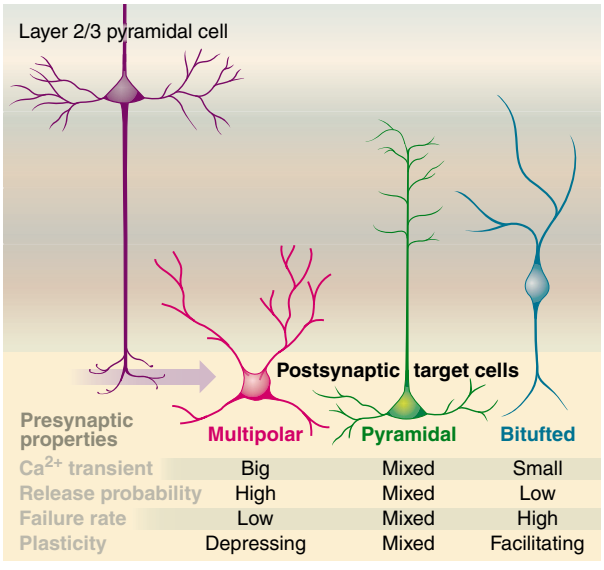
NEUROSCIENCE

Matching at the Synapse

Scott M. Thompson

One of the wonders of the brain is how its neurons organize themselves into complex synaptic networks. There are more cells in the mammalian brain than there are stars in the Milky Way. In the brain's cortex, each neuron forms synaptic contacts with as many as 10,000 target cells (*1*) that belong to five or more different cell types. The details of how these synaptic connections are constructed are important as synaptic strength governs the reliability of information transfer (through release of neurotransmitter) from the presynaptic to the postsynaptic neuron. On page 863 of this issue, Koester and Johnston (*2*) offer unexpected observations about just how precisely these synaptic connections are formed.

The strength of a synaptic connection depends on several key factors: the number of synaptic contacts formed between the two neurons (<10 in neocortex) (*3*), the number of neurotransmitter receptors expressed by the postsynaptic neuron, and the probability that an action potential will trigger the release (exocytosis) of neurotransmitter from the presynaptic nerve terminal into the synapse. The release probability of a given synaptic contact depends on the concentration of calcium ions (Ca^{2+}) in the presynaptic nerve terminal after arrival of an action potential and the sensitivity of the exocytotic machinery to the Ca^{2+} ion concentration.



Making connections. Dependence of presynaptic terminal properties on the type of postsynaptic target cell. Presynaptic boutons formed by the axons of layer 2/3 pyramidal cells of the rat somatosensory cortex form connections with three different classes of postsynaptic target cell (*2*). The three postsynaptic cell types include two classes of inhibitory interneurons, multipolar and bitufted, and pyramidal cells.

But what determines the probability of neurotransmitter release for each particular nerve terminal? Because of their small size, there are few synapses where release probability can even be measured. Working on the nerve terminals of rat neocortical pyramidal cells, Koester and Johnston now succeed in correlating the size of the transient increase in Ca^{2+} concentration evoked by the action potential with the probability of neurotransmitter release. They show that the properties of the presynaptic terminal responsible for neurotransmitter release differ depending on the type of postsynaptic target cell (see the figure).

To perform their technically superb studies, the authors made whole-cell recordings from 63 pairs of monosynaptically coupled neurons in ex vivo slices from the rat somatosensory cortex, maintained on the stage of a two-photon microscope. Presynaptic layer 2/3 pyramidal cells were loaded with the green Ca^{2+} indicator dye, OGB-1, and three different classes of postsynaptic target cells were visualized with the red dye Alexa-594. The authors then searched for and identified the small sites of synaptic contact between the two neurons. Measurements of the transient change in emission of the Ca^{2+} indicator dye provided an assay of the action potential–induced Ca^{2+} concentration in the nerve terminal.

The simplest outcome one might expect from such an experiment is that all of the presynaptic boutons (enlargements of the presynaptic nerve terminal from which neurotransmitter is released) of a given pyramidal cell would be essentially identical. The size of their Ca^{2+} transients would be dependent primarily on the level of voltage-dependent Ca^{2+} channel expression in that cell. There is, in fact, a 10-fold variation in the amplitudes of Ca^{2+} signals in the boutons from a single pyramidal cell (*4*). The surprising observation made by Koester and Johnston is that the size of the Ca^{2+} signal in a given bouton is not random, but rather is determined by the target cell with which that bouton forms a synapse. Ca^{2+} transients in pyramidal cell boutons apposed to one class of inhibitory interneuron that releases the

The author is in the Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201–1559, USA. E-mail: sthom003@umaryland.edu

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neurotransmitter γ-aminobutyric acid (GABA), the multipolar cell, were on average three times the size of Ca^{2+} transients in terminals apposed to another class of GABAergic interneuron, the bitufted cell. Pyramidal cell boutons apposed to other pyramidal cells displayed the full 10-fold range in amplitude of the Ca^{2+} transient.

What are the consequences of these differences in the amplitude of the Ca^{2+} transient? All else being equal, larger Ca^{2+} transients should produce greater increases in the probability of vesicle fusion with the presynaptic cell's plasma membrane and neurotransmitter release. Indirect analyses indicated that this was the case. The induction of an action potential in a pyramidal cell was more likely to trigger neurotransmitter release onto a multipolar cell than onto a bitufted cell. The authors also found an excellent correlation between the amplitude of the Ca^{2+} transient and the form of short-term synaptic plasticity displayed in response to brief trains of presynaptic action potentials, thus accounting for earlier observations (*5*). Pyramidal cell–to–bipolar cell connections were facilitated, whereas pyramidal cell–to–multipolar cell connections became depressed. Pyramidal cell–to–pyramidal cell connections varied, presumably depending on the initial probability of neurotransmitter release (*6*).

Koester and Johnston strengthened the correlation between the amplitude of the Ca^{2+} transient and release probability by introducing the Ca^{2+} indicator dye into postsynaptic cells and then assaying the probability that a presynaptic action potential in a pyramidal cell would cause postsynaptic Ca^{2+} influx via NMDA (*N*-methyl-D-aspartate) receptors. As expected from the relative amplitudes of the Ca^{2+} transients, the release probability was significantly higher at pyramidal cell boutons apposed to multipolar cells than at those apposed to bitufted cells.

The second surprising observation made by the authors is that not only are the Ca^{2+} transients and release probabilities uniform for different classes of target cells, but they are also uniform at multiple sites of contact between a single pair of cells. In 17 pairs, the authors were able to find two synapses between the pre- and postsynaptic cells. Both the amplitude of the Ca^{2+} transients in the two contacts and their respective release probabilities were found to be remarkably similar. Although this similarity might be expected for the synapses formed with the two classes of interneuron, which are relatively homogeneous with respect to both characteristics, it is remarkable for the pyramidal cell–pyramidal cell connections, whose values varied up to fourfold in this data set.

Koester and Johnston conclude with the provocative suggestion that there is communication between a pyramidal cell and each of its postsynaptic targets that serves to "normalize" the Ca^{2+} transients and release probabilities at all of the connections between the two cells. Neither the means of communication between the two cells nor the means of normalization are known. The distances between the various sites of contact between a given pair of cells can be quite large. Thus, the authors suggest that correlated pre- and postsynaptic discharges might serve as the signal, much as they do in long-term potentiation, the form of synaptic plasticity thought to underlie memory formation (*7*). This possibility is particularly intriguing for pyramidal cell–pyramidal cell connections, because there is good evidence that a change in release probability contributes to the expression of long-term potentiation in the neocortex (*8*). It is not clear how the presynaptic terminal would be aware of activity in the postsynaptic cell, but the process would seem to require a retrograde signal.

There are several potential means by which this signal could then modulate the size of the presynaptic Ca^{2+} transient, such as changing the density of Ca^{2+} channels, the subtype of the Ca^{2+} channels, the amount of endogenous Ca^{2+} buffer, or the probability of channel opening in response to depolarization. In the hippocampus, there is evidence of target cell–dependent expression of presynaptic inhibitory receptors (*9*, *10*), and this mechanism might also differentially regulate Ca^{2+} influx and release probability.

Like all good science, the observations reported by Koester and Johnston raise fascinating new questions. They also remind us of the wonder that is our brain.

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CELL BIOLOGY

Wnt Signaling Glows with RNAi

Eric R. Fearon and Ken M. Cadigan

Not long ago, scientists could only fantasize about insights that might be offered through high-throughput, comprehensive genetic screens that did not rely on characterization of discernible phenotypes in the whole animal. The pursuit of such screens presents several challenges, including the need for a priori knowledge about key factors in a pathway and for surrogate markers reflecting the pathway's status that can be rapidly assayed with robust results. Even with this functional screening approach in hand, the pursuit of high-throughput analyses of all genes in an organism requires a complete annotated genomic sequence and efficient RNA interference (RNAi) technologies for antagonizing gene function. Only recently has this type of screen been pursued (*1*–*4*). On page 826 of this issue, DasGupta, Perrimon, and their colleagues describe the application of RNAi methods to a *Drosophila* tissue culture system and report a plethora of new genes implicated in Wnt signaling (*5*).

Wnts are a conserved family of secreted proteins with varied and context-specific activities in embryonic and adult tissues. They mediate effects in numerous cellular processes including proliferation, survival, differentiation, and motility (*6*, *7*). Not unexpectedly, given the importance of Wnts in development and adult physiology, Wnt pathway defects have been implicated in human disease states, including many types of cancer as well as bone density and retinal vascular disorders (*6*, *7*). The application of classical genetic and biochemical methods to define major components of the Wnt pathway has yielded much information (see the figure). Wnts bind to a transmembrane receptor complex, and intracellular consequences are mediated via several distinct downstream pathways (see the figure). In the "canonical" Wnt pathway, β-catenin is the central player, and its levels and activity are tightly regulated by phosphorylation at certain serine and threonine amino acid residues in its amino terminus. Phosphorylated forms of β-catenin are preferentially ubiquitinated, then degraded by the protea-

The authors are at the Cancer Center, University of Michigan, Ann Arbor, MI 48109, USA; E. R. Fearon is also in the Departments of Internal Medicine, Human Genetics, and Pathology, and K. M. Cadigan is also in the Department of Molecular, Cellular, and Developmental Biology. E-mail: fearon@umich.edu

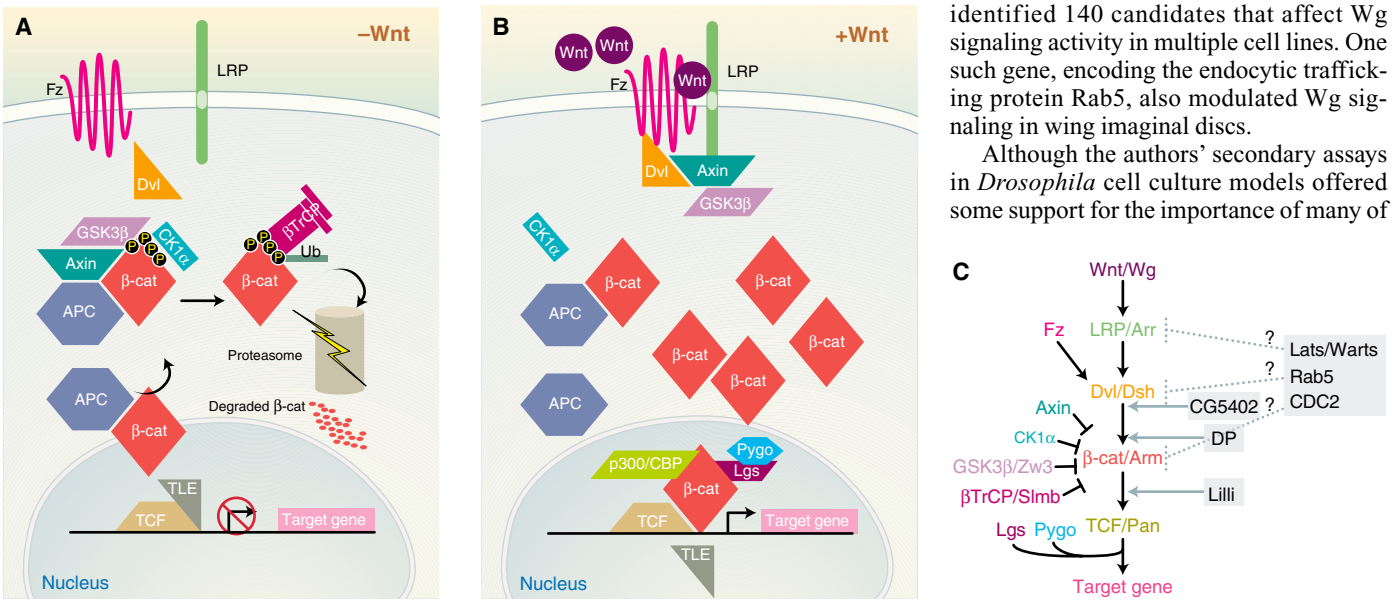
some. Activation of Wnt proteins inhibits β-catenin phosphorylation through poorly understood mechanisms. Wnt-mediated stabilization of the "free" pool of β-catenin leads to its accumulation in the nucleus and its enhanced binding to T cell factor (TCF) transcription factors. β-catenin–TCF complexes bind to specific DNA sequences in the regulatory regions of certain cellular genes and together with other nuclear cofactors, activate gene expression.

Despite much progress in defining factors in Wnt signaling by conventional approaches, key Wnt pathway components remain undiscovered. In genetic screens of *Drosophila* embryos, potential Wnt pathway genes may have escaped detection because they are expressed both maternally and in the zygote (*8*). Other genes might have been missed because they are essential for viability of the whole organism or because they operate both in the Wnt and other pathways. Most Wnt researchers predict the existence of additional Wnt pathway components, but few could have guessed at the 200 new candidate components that DasGupta *et al.* now report (*5*).

DasGupta *et al.*'s screening method used sensitive reporter genes containing multimerized TCF binding sites fused to minimal promoter elements upstream of a firefly luciferase gene. The screen was performed in the presence of exogenous Wingless (Wg, the archetypical *Drosophila* Wnt), so that the reporter assay had sufficient dynamic range to detect either inhibition or enhancement of the Wg-induced signal. The effect of ~22,000 duplex RNAs on the ability of Wg to activate luciferase expression was assessed in a *Drosophila* cell line derived from fruit fly imaginal discs. This collection of RNA duplexes corresponds to >95% of the genes known or predicted in the *Drosophila* genome. The primary screen was done in duplicate to minimize false positives, and 238 potential Wnt pathway genes were identified. The screen uncovered most, but not all, previously identified core Wnt pathway components. About half of the identified genes had a gene ontology annotation or recognizable predicted protein domain. The protein classes included transcription factors, kinases and phosphatases, ubiquitin ligases and proteasome components, heterotrimeric guanine nucleotide–binding proteins, and membrane proteins.

To characterize the genes further, DasGupta *et al.* exploited established consequences of gain- and loss-of-function mutations in well-characterized Wnt pathway components. For new genes whose functions are required for Wg signaling, the investigators determined where in the pathway the genes had their effects, using a double-stranded RNA (dsRNA) against each gene in combination with an expression construct that constitutively activates Wg signaling at a selected downstream point (for example, activation of LRP/Arr, Dsh, or Arm, the fruit fly β-catenin homolog). For genes that were apparent negative regulators of Wg signaling, combinations of dsRNAs were used. The results indicate that nearly all genes identified in the screen work upstream of β-catenin and TCF. When a primary screen identifies such a large number of genes, secondary screens become crucial in determining which genes are likely to be most important. The authors prioritized their list by examining the candidates in other contexts. By screening other *Drosophila* cell lines unrelated to the cell line used in the primary screen, the authors identified 140 candidates that affect Wg signaling activity in multiple cell lines. One such gene, encoding the endocytic trafficking protein Rab5, also modulated Wg signaling in wing imaginal discs.

Although the authors' secondary assays in *Drosophila* cell culture models offered some support for the importance of many of



Selected Wnt pathway components, the old and the new. (**A**) Regulation of β-catenin (β-cat) in the absence of Wnts. Glycogen synthase kinase 3β (GSK3β) works together with the Axin and APC tumor suppressor proteins and perhaps other factors, such as casein kinase 1α (CK1α). This complex phosphorylates β-catenin at multiple serine and threonine residues in its amino terminus. Phosphorylated β-catenin is ubiquitinated by cellular β-transducin repeat-containing proteins (βTrCPs) and then degraded by the 26*S* proteasome. Thus, β-catenin is no longer able to bind to TCF transcription factors. The latter are free to interact with TLE (transducin-like enhancer of split) proteins, resulting in transcriptional repression of target genes. (**B**) Binding of activated Wnts to the Fz-LRP coreceptor inhibits the GSK3β–Axin complex by uncertain mechanisms that may involve inhibition of the complex by disheveled (Dvl) and LRP's recruitment of Axin to the plasma membrane. The net consequence is the stabilization of the "free" pool of β-catenin, leading to its accumulation in the nucleus, its binding to TCF, and the displacement of TLE. The β-catenin–TCF complex regulates transcription in concert with cofactors such as p300/CBP and the Pygo protein, with Pygo recruitment occurring via its interaction with the β-catenin–binding protein legless (Lgs). (**C**) Interactions among known Wnt components and new candidate components (*5*). Where multiple names for components exist, a mammalian designation is indicated first, followed by the *Drosophila* designation (Wg, Wingless; Arr, Arrow; Dsh, disheveled; Zw3, Zeste white 3; Arm, Armadillo; Slmb, Slimb; Pan, pangolin). Positive activities in Wnt/Wg signaling are indicated by arrows; inhibitory effects are indicated by bars. Three candidate positive regulators are indicated (CG5402; DP, dimerization partner; Lilli, lilliputian), along with their apparent points of interaction in the pathway, based on epistasis studies. Three candidate negative regulators are also indicated: CDC2, cyclin-dependent kinase 2; Rab5; Lats, large tumor suppressor (called Warts in *Drosophila*). It is uncertain at which point in the pathway the negative regulators operate.

the genes uncovered, a skeptic might still judge the screen and its results to have uncertain physiological significance without additional data. In bolstering the case for selected gene candidates, DasGupta and co-workers made a wise choice in exploiting genetic approaches to pursue the notion that some genes uncovered by the screen will have conserved Wnt signaling function in multiple species and models. (Models included reporter gene assays in mammalian cultured cells, development of *Drosophila* wing imaginal discs, and zebrafish morphogenesis.) Indeed, the authors demonstrate the importance of several new Wnt pathway components—Rab5, the serine-threonine kinase Lats/Warts, and a pairlike homeobox gene (CG4136)—in multiple settings.

Almost certainly, a subset of candidate Wnt pathway components identified by DasGupta *et al.* will be major players in one or more cell types, although many candidates may regulate the Wnt pathway indirectly. No doubt it will take Wnt researchers time to assess the in vivo relevance of the candidate Wnt pathway genes highlighted by the study and to determine their biochemical connection to the pathway. The list of candidate Wnt pathway components generated by DasGupta *et al.* is remarkable, but it is certainly not complete. For example, some components known to act redundantly, such as APC1 and APC2 (*9*, *10*), were missed. Also, the model reporter genes used in the screens bear minimal similarity to endogenous genes dependent on β-catenin–TCF complexes. Thus, the screens may be more informative about factors acting upstream of Arm/β-catenin. However, given that straightforward modifications can be made to reporter gene constructs and assays, it should be easy to develop additional screens to fill apparent gaps. Among the major challenges will be integrating the abundance of candidate components into useful working models of Wnt pathway function. Given the importance of Wnts in development and disease, future studies are sure to identify links between some of the new pathway components and essential developmental processes and diseases such as cancer. Such discoveries will only serve to increase the glow surrounding the Wnt signaling pathway.

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RETROSPECTIVE

Stanley Joel Korsmeyer (1950–2005)

Timothy J. Ley

Stanley Korsmeyer died from lung cancer on 31 March 2005, at the age of 54. He had never smoked. He grew up on the family farm in southwestern Illinois. Given his farming roots, he thought about becoming a veterinarian. But one of his early mentors, a local vet named Robert Goodin, advised him to think more about a career in biological sciences. What an incredibly good piece of advice that would turn out to be. Stan didn't really know how to pursue such a goal—he had no role models and no connections. But he had desire and determination, and a very supportive family. His parents prepared him for life with the bedrock values of farming: thoughtful preparation, hard work, personal integrity, and neighborly kindness. These values would serve him well throughout his life.

After majoring in biology at the University of Illinois at Urbana-Champaign, Stan went to the University of Illinois medical school in Chicago. Here, he met the great hematologist Paul Heller, who recognized Stan's potential. Heller encouraged Stan to pursue a research career and facilitated his first research experience, with Robert Strickland at the University of New Mexico. Stan studied lymphocytotoxic antibodies in the families of patients with inflammatory bowel disease and, while still a medical student, was the first author of a *New England Journal of Medicine* paper. His love of immunology was set in stone.

While a medical resident at the University of California, San Francisco, he met a gifted oncology nurse named Susan Reynard, whom he married. In 1979, Stan joined the laboratory of Tom Waldmann at the National Institutes of Health. In a spectacular collaboration with Phil Leder's group, Stan and his colleagues defined immunoglobulin gene rearrangements in normal and diseased B lymphocytes. Using the molecular reagents generated by these studies, Bakhshi and Korsmeyer described the breakpoint region of a translocation between chromosomes 14 and 18 that is found in most follicular lymphomas. The translocation juxtaposed regulatory elements from the immunoglobulin locus with a previously undescribed gene, which they called *Bcl-2*. The *Bcl-2* gene was not altered in any obvious way, suggesting that its expression was simply dysregulated in B cell lymphomas. However, this gene did not resemble traditional oncogenes, and its link to pathogenesis was uncertain.

In 1986, Stan moved his laboratory to the Department of Medicine at Washington University medical school in St. Louis, where he became a Howard Hughes Investigator. He set up a highly focused and robust laboratory within a year, and began to attract the best and the brightest. His drive and focus were nothing short of incredible. Within a few years, Stan and his colleagues made transgenic mice that overexpressed the Bcl-2 protein in B lymphocytes. These animals developed follicular hyperplasia not because of excessive B cell proliferation, but rather because the B cells failed to die on schedule. These long-lived B cells went on to acquire additional mutations that ultimately led to the development of high-grade lymphomas in mice. This finding demonstrated that "wild-type" Bcl-2 could prevent cell death and lead to the development of cancer. The link with cancer was expected, but the mechanism was not. In the mid-1980s, cancer was thought to be a disease of increased cellular proliferation. The idea that it might be caused by reduced cell death was not

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The author is in the Departments of Medicine and Genetics, Washington University, St. Louis, MO 63110, USA. E-mail: tley@im.wustl.edu

recognized. The data from Stan's group were compelling and incontrovertible, launching Bcl-2 as the founding member of a new class of oncogenes. The earlier proliferative paradigm of cancer pathogenesis was not wrong, but was simply incomplete. Dysregulated programmed cell death would soon be demonstrated in many tumors, and the word "apoptosis" would become part of the vernacular for all biomedical scientists.

For the rest of his life, Stan embraced the key scientific question posed by these studies: How does Bcl-2 block programmed cell death? He and his colleagues defined the physiological roles of Bcl-2 in B cell memory and T cell development, and showed that this protein was required for the survival of many cell types during normal development. Stan and his collaborators demonstrated that Bcl-2 is only one member of a large group of related proteins with conserved homology domains. Moreover, he and others showed that these proteins interact and subserve both pro- and antiapoptotic functions that regulate cell survival by affecting critical mitochondrial functions.

For these many remarkable observations, Stan was elected to the National Academy of Sciences at the age of 45. He proceeded to win the Bristol-Myers Squibb Award, the Mott Prize of the General Motors Cancer Research Foundation, the Pezcoller Foundation–American Association for Cancer Research International Prize, and the Stratton Medal from the American Society of Hematology, to name but a few of his many awards. David Nathan and the leadership at the Dana-Farber Cancer Institute recruited him to Harvard in 1998. There, he continued his extraordinary science and acted as a senior scientific leader of the institution until his untimely death.

Stan was one of the most highly cited scientists of our time. He published more than 250 peer-reviewed papers that were cited, in total, more than 40,000 times. Remarkably, 23 of his publications were cited at least 500 times; 11 were cited more than a thousand times. His papers reflect his experimental precision and creative genius; they were impeccably edited, understated, and a joy to read.

Stan's most enduring scientific legacy—and the one of which he was proudest—was that of his trainees. Forty of his former postdoctoral fellows now hold faculty positions at universities around the world. Stan never ran a mega-lab, because he worried too much about the well-being of every person that he mentored. When a graduate student told Stan that he was struggling, Stan smiled and replied, "Okay, let's struggle together," and he meant it. He brought out the best in every person he trained, and he served as a wonderful role model for future generations of physician-scientists. Most appropriately, he won the Barger Award for Excellence in Mentoring at Harvard last year.

A spirit of caring and humility pervaded all that Stan did. Despite his many scientific accolades, his source of greatest pride was his family. His wife of 25 years, Susan, and his sons, Jason and Evan, were the most important people in his life. The lessons of his parents and the farm in Beardstown, Illinois, were never far from his mind, and they kept him grounded. Although he was a visionary scientist and a natural leader, he was even more so a compassionate human being whose mission was to heal. He had an ever-optimistic view of life, and a broad, genuine smile that could light up a room. He embodied the spirit of Wordsworth, who wrote: "That best portion of a good man's life, his little, nameless, unremembered acts of kindness and of love." To Stan Korsmeyer, that was the best portion indeed.

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ATMOSPHERE

Air Pollution–Related Illness: Effects of Particles

André Nel

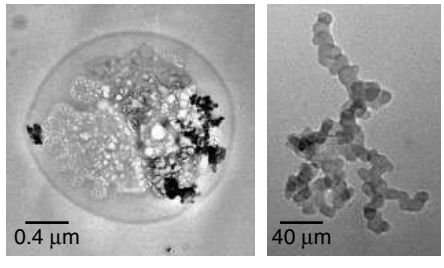
Worldwide epidemiological studies show a consistent increase in cardiac and respiratory morbidity and mortality from exposure to particulate matter (PM) (*1*–*3*). PM is a key ingredient of polluted air and is estimated to kill more than 500,000 people each year (*4*). To prevent this staggering loss of life we must understand the characteristics of the toxic particles and gain insight into how these characteristics are related to adverse health effects (*5*). As our understanding increases, we can use this knowledge to develop biomarkers in the hope of identifying susceptible individuals and reducing their exposure to PM.

Enhanced online at www.sciencemag.org/cgi/content/full/308/5723/804

PM is composed of solid and liquid particles that come from sources such as vehicle exhaust, road dust, smokestacks, forest fires, windblown soil, volcanic emissions, and sea spray (*6*). Particle size, surface area, and chemical composition determine the health risk posed by PM (*7*). PM can be classified into coarse, fine, or ultrafine particles (*6*). Coarse particles, which have a diameter of more than 2.5 μm, are mostly derived from soil and sea salts. Fine particles (0.1 to 2.5 μm in diameter) and ultrafines (<0.1 μm in diameter) are predominantly derived from combustion of fossil fuel (see the first figure). Combustion particles have a core of elemental carbon that is coated with a layer of chemicals, including organic hydrocarbons, metals, nitrates, and sulfates. All of these components may play a role in particle toxicity (*7*).

Currently, government and air-quality monitoring agencies track and regulate 10-μm-diameter (PM10) and 2.5-μm-diameter (PM2.5) particles. Unfortunately, the unregulated ultrafine particles are potentially the most dangerous. Ultrafines are the major component in vehicle emissions—the largest source of air pollution in urban areas (*8*)—and they have the largest surface area and highest content of potentially toxic hydrocarbons among all PM sources. They can also penetrate deeper into lung tissue than fine or coarse particles (*8*).

Pulmonary effects of PM include the triggering of inflammation in the smaller airways, which can lead to the exacerbation



Dangerous dirt. (**Left**) Electron micrograph of a fine mode particle collected by an impactor from air outside an engineering laboratory at the University of California, Los Angeles. A halo surrounds residues of what are probably inorganic salts and polar organic compounds dissolved in the original aqueous droplet. Sootlike particles are also present. (**Right**) Aggregates of ultrafine particles collected on the last stage of an eight-stage impactor. These are soot particles emitted from diesel engine sources such as buses. More volatile particles may have evaporated in the electron microscope.

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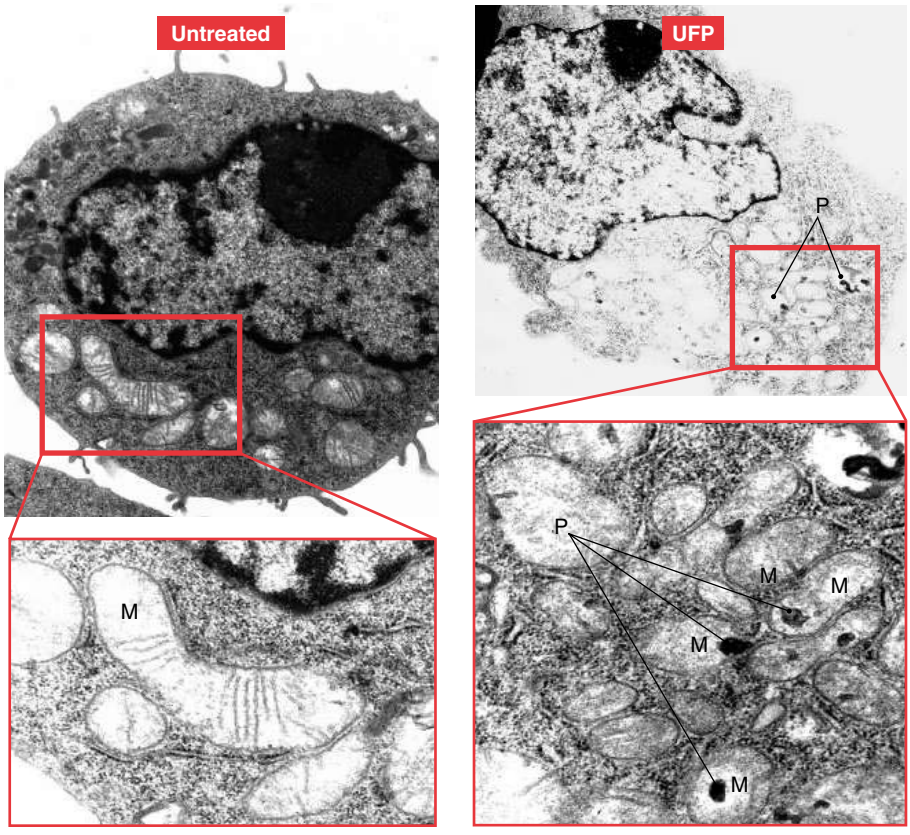
The author is in the Department of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA. E-mail: anel@mednet.ucla.edu



Toxic particles. The effect of ultrafine particles (UFP) in a macrophage cell line. (**Left**) An untreated macrophage with healthy mitochondria (M). (**Right**) The same cell type treated with ambient ultrafine particles, collected in the Los Angeles basin. The enlarged images show that the untreated cell has healthy mitochondria with cristae, whereas the treated cell has damaged mitochondria that lack cristae. The vacuolar structures in the treated cell each represent a mitochondrion with included particles (P). Whether the particles gain access to and then damage the mitochondria or gain access to already damaged mitochondria is unknown. [Modified from (*15*)]

of asthma and chronic bronchitis, airway obstruction, and decreased gas exchange (*1*, *2*, *9*). PM can also interfere with the clearance and inactivation of bacteria in lung tissue. More recently, there has been a growing awareness that PM is a cardiovascular risk factor that is associated with heart attacks, stroke, heart rhythm disturbances, and sudden death (*3*).

A number of mechanisms have been proposed to explain the adverse health impact of PM (*5*). Effects of PM that have experimental support are inflammation, cytokine and chemokine release, production of white blood cells, oxygen free-radical production in the lungs, endotoxin-mediated cellular and tissue responses, stimulation of irritant receptors, and covalent modification of key cellular enzymes (*5*, *9*). Best characterized in humans are the effects of PM on airway inflammation (*10*). In human and animal studies, inhalation of particles elicits proinflammatory effects, cytokine production, and enhancement of allergic responses in the upper and lower airways (*9*–*11*). PM exposure is likely linked to inflammation through the generation of reactive oxygen species and oxidative stress (*9*, *12*–*14*). Although there is still debate about which particle components are responsible for producing reactive oxygen species, there is accumulating evidence that pro-oxidative organic hydrocarbons, such as polycyclic aromatic hydrocarbons and quinones, and transition metals, such as copper, vanadium, chromium, nickel, cobalt, and iron, play a role (*15*, *16*). The particle provides a template for electron transfer to molecular oxygen in these reduction and oxidation (redox) cycling events (*7*). In addition, target cells, such as airway epithelial cells and macrophages, generate reactive oxygen species in response to particle uptake by biologically catalyzed redox reactions that occur in the cell membrane and mitochondria (*9*, *13*, *15*). The second figure shows mitochondrial damage to a macrophage caused by ultrafine particles.

Reactive oxygen species can damage cellular proteins, lipids, membranes, and DNA. To defend against this damage, cells use up their stores of a key antioxidant, glutathione. The glutathione depletion can induce a state of cellular stress, called oxidative stress, that triggers an increase in the production of antioxidant enzymes through activation of a transcription factor Nrf2 (*17*). Failure to overcome oxidative stress leads to the activation of additional intracellular signaling cascades that regulate the expression of cytokine and chemokine genes (*14*, *16*). These products are produced locally in target tissues as well as systemically, and lead to widespread proinflammatory effects remote from the site of damage.

Some individuals may be more prone to the development of inflammation, asthma, and allergic responses, because of mutations in the genes involved in the induction of the antioxidant defense (*18*). Other conditions that predispose to PM susceptibility include old age, preexisting chronic heart and lung disease, and diabetes mellitus, all of which are associated with oxidative stress and inflammation.

Although oxidative stress and inflammation may explain aspects of cardiovascular disease such as the growth of atherosclerotic plaques, other adverse outcomes, such as sudden death, may result from altered autonomic regulation of heart rate and changes in the clotting abilities of the blood (*3*). Although the cause of altered autonomic nervous activity is unknown, the systemic release of cytokines from the lung and vasculature may affect the production of clotting factors and anticoagulant enzymes in the liver. This could lead to the formation of a dense clot on top of a ruptured atherosclerotic plaque, the pathological hallmark of fatal heart attacks. The role of adsorbed particle chemicals in these cardiovascular events is uncertain. However, it is noteworthy that the ultrafine particles may gain access to the systemic circulation by penetrating alveolar membranes in lung tissue (*19*).

Public concern about the adverse health impact of PM should drive future research. We need to determine which chemical components are most important and whether, in addition to the PM mass, we also need to monitor particle number when considering the effects of ultrafine particles. Products of oxidative stress, inflammation, or tissue damage can be used as biomarkers for early indication of adverse effects of PM exposure. These biomarkers could be monitored in population studies to find susceptible subsets and to determine whether regulatory efforts are sufficient to protect against PM-induced or PM-exacerbated disease.

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ATMOSPHERIC SCIENCE

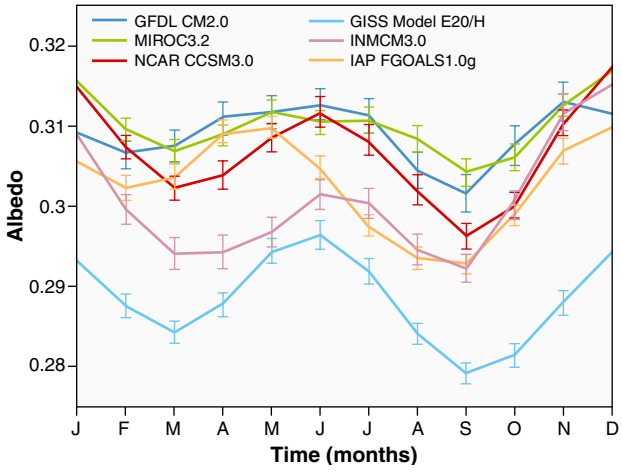
In Search of Balance

Robert J. Charlson, Francisco P. J. Valero, John H. Seinfeld

The climate of Earth and its global mean surface temperature are the consequence of a balance between the amount of solar radiation absorbed by Earth's surface and atmosphere and the amount of outgoing longwave radiation emitted by the system. The former is governed by the albedo (reflectivity) of the system, whereas the latter depends strongly on the atmospheric content of gases and particles (such as clouds and dust). Although the theory of absorption of infrared radiation by gases in the atmosphere (*1*) is well accepted and embodied in climate models, the observational and theoretical treatments of albedo, aerosols, and clouds are still under development. One brevium (*2*) and two reports (*3*, *4*) in this issue report estimates of Earth's albedo and of solar radiation reaching the surface, but the uncertainties remain large.

The buildup of CO_2 (*5*), CH_4, and other greenhouse gases during the past century has led to an increased absorption of infrared radiation in the atmosphere (enhanced greenhouse effect) and a consequent warming ("positive forcing") of the climate. But human-made changes in aerosols and clouds can cause enhanced albedo and hence cooling ("negative forcing"), and they may already have offset a substantial part of the enhanced greenhouse effect. Present trends suggest that by 2050, the magnitude of the enhanced greenhouse effect will be so large that the net anthropogenic forcing will be unequivocally positive and substantial in magnitude (*6*).

Changes in energy balance affect a host of climatic factors, such as temperature, sea level, meteorological patterns, and precipitation. To understand and quantify these effects, the enhanced greenhouse effect and all other forcings must be known accurately. To complicate matters further, the enhanced greenhouse effect is suspected of causing changes in clouds and hence albedo, resulting in feedbacks on both incoming and outgoing radiation (*7*).



Apparent agreement. Monthly mean annual cycle and standard deviation (vertical bars) of albedo from six models (*12*, *15*). These and other models are used by the Intergovernmental Panel on Climate Change (IPCC) for preindustrial control simulations.

Increased albedo could counteract the enhanced greenhouse effect on a global scale. However, the spatial and temporal characteristics of aerosols, clouds, and greenhouse gases differ widely. Clouds change rapidly, and atmospheric residence times for aerosols are short relative to those for the key greenhouse gases (which remain in the atmosphere for centuries). Albedo therefore changes rapidly, whereas the enhanced greenhouse effect simply increases as a result of the slow accumulation of greenhouse gases. Local and regional changes in energy balance would occur even if the albedo change could offset the enhanced greenhouse effect globally. Light-absorbing aerosols further complicate the picture by cooling Earth's surface, heating the atmosphere, and making clouds more absorbing; they may even reduce cloud cover, thereby decreasing albedo further.

These considerations underscore the importance of understanding the natural and anthropogenic changes in Earth's albedo and the need for sustained, direct, and simultaneous observations of albedo with all methods that are currently available. Albedo changes may be as important as changes in greenhouse gases for determining changes in global climate.

Many methods have been used to estimate albedo, which cannot be measured directly. These methods differ in their scattering geometries, calibration accuracy, and in spectral, space, and time coverage. The different modes of observation include measurements of earthshine reflected from the Moon (*8*, *9*), broadband radiometer data from low orbits around Earth [Wielicki *et al.* on page 825 (*2*)], geostationary cloud-cover observations (*10*), deep space radiometry (*11*), and surface radiometry [Pinker *et al.* on page 850 (*3*), Wild *et al.* on page 847 (*4*)]. All these methods require a theoretical model for relating the measured parameters to albedo, and they all rely on different assumptions. It is critical to compare the results from different approaches to test the consistency among them.

The scientific community has recognized this essential need for years, but major impediments have developed. For example, the broadband data collected by the ERBS (Earth Radiation Budget Satellite) between 2000 and 2004 are not being analyzed for budgetary reasons. The DSCOVR (Deep Space Climate Observatory) satellite has been built but has since fallen victim to the delayed space shuttle program and is now in storage awaiting a launch opportunity. The CALIPSO (Cloud-Aerosol Lidar and Infrared Pathfinder Satellite Observation) and CloudSat satellites have been built and have scheduled launches, but recent budget cuts imposed on the Earth sciences in NASA will severely constrain the analysis and interpretation of the data. Inasmuch as

R. J. Charlson is in the Department of Atmospheric Sciences and Department of Chemistry, University of Washington, Seattle, WA 98195, USA. F. P. J. Valero is in the Center for Atmospheric Sciences, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093, USA. E-mail: fvalero@ucsd.edu J. H. Seinfeld is in the Department of Chemical Engineering and the Department of Environmental Science and Engineering, California Institute of Technology, Pasadena, CA 91125, USA. E-mail: seinfeld@caltech.edu

LARGE INCONSISTENCIES

Climatic observations and forcings	Equivalent change in albedo × 10^3
Enhanced greenhouse effect during industrial era (2.4 ± 0.2 W/m^2) (*6*)	–7 ± 0.6
Anthropogenic aerosol forcing during industrial era (*6*)	+4 ± 4
Albedo change estimated from earthshine data (2000 to 2004) (*2*, *8*, *9*)	+16
Albedo change estimated from low-orbit satellite data (2000 to 2004) (*2*)	–6
Change in irradiance at Earth's surface measured with satellites (1983 to 2001) (*3*)	–8
Change in irradiance at Earth's surface measured at the surface (1985 to 2000) [Fig. 1 in (*4*)]	–13
Change in irradiance at Earth's surface measured at the surface (1950 to 1990) [Fig. 1 in (*4*)]	+20

the primary objectives of these three satellites include studies of the effects of aerosols and clouds on albedo, what seemed to be real progress could be delayed or thwarted.

Several global climate models appear to calculate nearly the same albedo (see the figure); however, clouds are treated very differently in these models, the seasonal cycles that are prominent in the figure are not apparent in data from the CERES (Clouds and the Earth's Radiant Energy System) experiment or from earthshine data (*2*, *8*, *9*, *12*), and the global amount of condensed water varies among the models by as much as a factor of 5. Hence, little certainty can be gained from models alone.

To date, the results from different measurement and modeling approaches are inconsistent among themselves and with each other. The magnitudes of the inconsistencies exhibited by both measurements and models of albedo changes and effects are as large as, or larger than, the entire enhanced greenhouse gas effect when compared in terms of the albedo change equivalent of climate forcing (see the table). In fact, the albedo change that is the equivalent of the enhanced greenhouse effect is barely detectable by the available methods for measuring albedo.

To quantify all changes in energy balance, and in view of the discrepancies in magnitude and even sign (see the table and the figure), it will be necessary to develop a strategy to strengthen research efforts on albedo-related quantities, including modeling and analysis of the data from the yet-to-be-launched satellites. To help achieve a balance of effort, care must be exercised in the use of potentially misleading terms like "global warming" (*13*) and "global dimming" (*14*). Their use may constitute an obstacle in reaching an understanding of the issues driving the fundamental scientific questions of Earth's energy balance, albedo, greenhouse effect, and interactions of solar and infrared radiation with aerosols and clouds.

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13. Global warming formally means an increase in the mean temperature at Earth's surface, but may seem to imply (incorrectly) that the whole Earth will warm more or less uniformly.
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15. GFDL: Geophysical Fluid Dynamics Laboratory, USA. NCAR: National Center for Atmospheric Research, USA. GISS: Goddard Institute for Space Studies, USA. INMCM: Institute for Numerical Mathematics, Russia. IAP: Institute of Atmospheric Physics, China. Miroc is a medium-resolution model run by the Center for Climate System Research (University of Tokyo), the National Institute for Environmental Studies, and the Frontier Research Center for Global Change of the Japan Agency for Marine-Earth Science and Technology.

16. We thank the international modeling groups for providing their data for analysis, the Program for Climate Model Diagnosis and Intercomparison for collecting and archiving the model data, the JSC/CLIVAR Working Group on Coupled Modelling and their Coupled Model Intercomparison Project and Climate Simulation Panel for organizing the model data analysis, and the IPCC Working Group I Technical Support Unit for technical support. The IPCC Data Archive at Lawrence Livermore National Laboratory is supported by the Office of Science, U.S. Department of Energy.

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GEOCHEMISTRY

The Paradox of Mantle Redox

Catherine McCammon

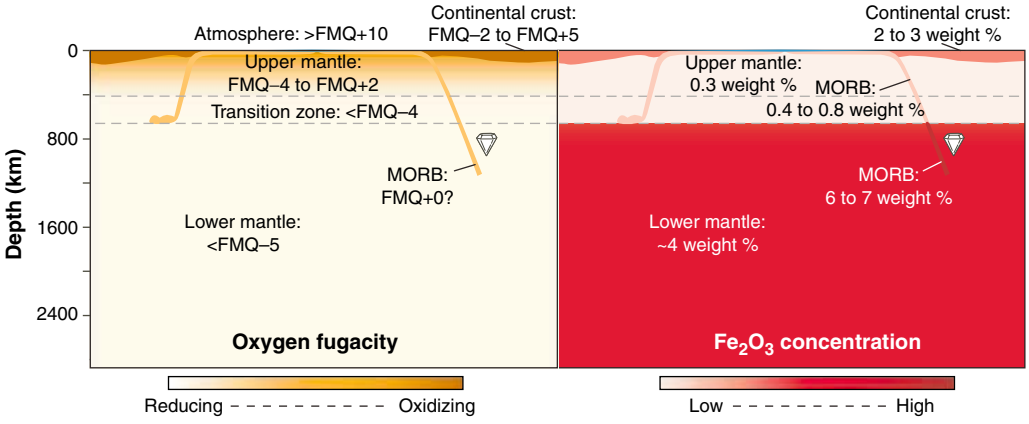
Redox reactions (those involving reduction or oxidation) occur in many everyday processes, from photosynthesis and metabolism to fuel combustion and household cleaning. They also play a critical role in many geological systems. Processes on Earth's surface are intimately linked to the oxidation state of the mantle through the geochemical cycles of elements such as carbon, sulfur, oxygen, and hydrogen. Recent studies have advanced our understanding of the oxidation state of the mantle, elucidating the redox relations within Earth and their consequences for global processes.

The author is at Bayerisches Geoinstitut, Universitaet Bayreuth, Bayreuth, 95440 Germany. E-mail: catherine.mccammon@uni-bayreuth.de

The term "oxidation state" has caused some confusion in the geological literature, because it has two different meanings in the context of mantle properties. First, it is used to indicate the valence state of elements, for example, divalent iron (Fe^{2+}) and trivalent iron (Fe^{3+}). Second, it is used to indicate the chemical potential of oxygen, more commonly referred to as oxygen fugacity. High oxygen fugacity means oxidizing conditions, whereas low oxygen fugacity implies reducing conditions.

In everyday experience, these two definitions of oxidation state are almost always coupled: Oxidizing conditions favor the formation of Fe^{3+} (for example, rust on a car), whereas reducing conditions favor the formation of Fe^{2+} or even metallic iron (Fe^0). However, paradoxical behaviors can arise when solids are present, because crystal structures impose additional constraints: Some minerals incorporate almost no Fe^{3+} even under oxidizing conditions, whereas others incorporate Fe^{3+} even under reducing conditions. A classic example is iron oxide, Fe_xO, which always contains a measurable amount of Fe^{3+} in its crystal structure, even under reducing conditions where metallic iron is stable.

Studies of mantle rocks show that the oxygen fugacity of the upper mantle is relatively high (*1*), even though the abundance of oxidized iron (Fe^{3+}) is low (*2*) (see the figure). How can we reconcile these apparently contradictory observations? The answer lies in the unfavorable energetics of defect incorporation in olivine, the most abundant mineral in the upper mantle. This property leads to an almost negligible Fe^{3+} concentration in olivine even under relatively oxidizing conditions (*3*). Fe^{3+} is readily incorporated into the minerals spinel and garnet, but because they are at least 1/10th as abundant as olivine, their presence causes only a small increase in Fe^{3+}



The oxidation state of Earth's mantle. The paradoxical relation between oxygen fugacity (**left**) and Fe^{3+} abundance (**right**) arises from the aversion of upper-mantle olivine and the strong affinity of lower-mantle perovskite for Fe^{3+}. Oxygen fugacity is expressed relative to the conditions where fayalite, magnetite, and quartz are in equilibrium (the FMQ buffer); positive values are more oxidizing, and negative values are more reducing. Fe^{3+} concentrations are expressed as weight % Fe_2O_3. The high Fe^{3+} concentration of the lower mantle is balanced by ~1 weight % metallic iron (*6*). Diamond formation in the lower mantle may arise from redox gradients in subducted material (*18*); other heterogeneities in mantle oxidation state are likely but not shown. The composition of subducted slabs is simplified to that of mid-ocean ridge basalt (MORB). Numerical estimates of oxygen fugacity and Fe^{3+} abundance are from (*7*, *15*).

abundance. On the other hand, the Fe^{3+} concentrations of spinel and garnet determine oxygen fugacity through mineral equilibria (*1*); the resulting oxygen fugacity of the upper mantle is therefore relatively high.

The relation between Fe^{3+} abundance and oxygen fugacity is also paradoxical in the lower mantle, but in an opposite sense. For decades it was assumed, based on the behavior of the upper mantle, that the dominant phase of the lower mantle, magnesium silicate perovskite, contains iron primarily as Fe^{2+}. But high-pressure experiments on perovskites containing aluminum (a small amount of which is believed to be present in the lower mantle) have shown this assumption to be wrong (*4*). Subsequent studies, including those on natural samples (*5*), have confirmed that the combined substitution of Al^{3+} and iron into magnesium silicate perovskite stabilizes Fe^{3+}. Fe^{3+} concentrations are high even under extremely reducing conditions (*6*).

The high Fe^{3+} concentration in lower-mantle magnesium silicate perovskite has at least two important consequences. First, the Fe^{3+} concentration in the dominant lower mantle phase cannot be ignored; about 50% of the iron in the perovskite is expected to be Fe^{3+}. The properties of the lower mantle may therefore differ from those predicted by aluminum-free experiments.

The difference between Fe^{2+} and Fe^{3+} in mantle minerals goes beyond the removal of an electron. In mantle minerals, iron occurs most frequently as Fe^{2+}, and can thus substitute for Mg^{2+} without affecting the charge balance of an individual crystal. In contrast, substitution of Fe^{3+} causes an initial charge imbalance, because there are no other abundant elements with the same charge. Balancing the charge requires effects such as coupled substitution (for example, $2Fe^{3+}$ for $Mg^{2+} + Si^{4+}$), the creation of defects, and/or the addition or loss of volatile elements such as hydrogen. Many mantle properties are sensitive to such effects; electrical conductivity, elasticity, and trace-element partitioning have been found to vary substantially with trivalent cation (Fe^{3+}, Al^{3+}) concentration (*7*).

The second consequence of high Fe^{3+} in lower-mantle magnesium silicate perovskite is that oxidation of iron must be balanced by a corresponding reduction reaction. The most likely reaction is self-reduction of iron according to $3Fe^{2+} \rightarrow Fe^0$ (metal) + $2Fe^{3+}$, which results in the production of ~1 weight % metallic iron in the lower mantle (*6*).

Although such a metal phase would probably evade geophysical detection, the potential geochemical consequences are immense, including the resolution of two long-standing conundrums: how the upper mantle became oxidized, and why the abundance of siderophile (metal-loving) elements in the mantle is so high (*6*).

The evolution of the oxidation state of the mantle through time has been a focus of hot debate, particularly because it relates to the rise of atmospheric oxygen and the origin of life (*8*). The abundance of redox-sensitive trace elements provides a window to the ancient mantle. Results have shown that the oxidation state of the upper mantle has remained essentially constant for ~3.5 billion years (*9*, *10*), implying that the upper mantle became oxidized relatively early in its history.

One plausible scenario for this oxidation is the removal during core formation of ~10% of the metallic iron that formed through self-reduction when magnesium silicate perovskite first became stable within the accreting Earth (*6*). The removal of 10% of this metallic iron from the lower mantle would cause a net increase in Fe^{3+} in the lower mantle that, following mantle convection, would raise the oxygen fugacity of the upper mantle to current values. If the remaining metallic iron took up a portion of the siderophile elements, the redistribution of these elements throughout the mantle could account for their unusually high abundance (*11*) and remove the need for a late-stage addition of meteoritic material to the accreting Earth (*12*).

The mantle is connected to the atmosphere and the hydrosphere through geochemical cycles of the volatile elements. Subduction of oxidized sediments and exhalation of volcanic gases are a driving force in these cycles (*13*), but the nature of their coupling to the oxidation state of the mantle remains unclear.

Techniques to measure the oxidation state have improved rapidly in the recent past as a result of increased spatial resolution and the development of new proxies for oxygen fugacity. Coupled with focused research initiatives (*14*), the use of these techniques should improve our knowledge of the temporal and spatial evolution of the oxidation state of the mantle. Such information is crucial to understanding the rise in atmospheric oxygen that occurred ~2.3 billion years ago (*15*), and may reveal whether atmospheric oxygen and perhaps life itself can be attributed to the peculiar properties of the perovskite structure.

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INTRODUCTION

All for One and One for All

As scientific instruments become ever more powerful, from orbiting observatories to genome-sequencing machines, they are making their fields data-rich but analysis-poor. Ground-based telescopes in digital sky surveys are currently pouring several hundred terabytes (10^{12} bytes) of data per year into dozens of archives, enough to keep astronomers busy for decades. The four satellites of NASA's Earth Observing System currently beam down 1000 terabytes annually, far more than earth scientists can hope to calibrate and analyze. And looming on the horizon is the Large Hadron Collider, the world's largest physics experiment, now under construction at CERN, Europe's particle physics lab near Geneva. Soon after it comes online in 2007, each of the five detectors will be spewing out several petabytes (10^{15} bytes) of data—about a million DVDs' worth—every year.

These and similar outpourings of information are overwhelming the available computing power. Few researchers have access to the powerful supercomputers that could make inroads into such vast data sets, so they are trying to be more creative. Some are parceling big computing jobs into small work packages and distributing them to underused computers on the Internet. With this strategy, insurmountable tasks may soon become manageable.

One approach to such "distributed computing" was pioneered by computer scientists working with SETI, the Search for Extraterrestrial Intelligence. The phenomenally successful SETI@home program now makes use of the idle computer time of millions of ordinary computer users, working as a screen saver to quietly crunch away at radio-signal data from deep space. As John Bohannon describes on p. 810, the same screensaver technique is now being used by a wide array of researchers studying everything from climate change to gravitational waves and protein folding. Bohannon also delves into the strange tribal world (p. 812) of the "crunchers": computer enthusiasts whose goal is to become the most prolific processors of data for various screen-saver research projects. And on p. 813, Mark Buchanan samples a piece of computer navel gazing: a distributed computing project to study the geography of the Internet itself.


PAGE 813

Another way of distributing both data and computing power, known as grid computing, taps the Internet to put petabyte processing on every researcher's desktop. On p. 814, Foster highlights the development of a lingua franca of grid computing: a set of standardized interfaces and protocols that permits researchers to work across the Web. Hey and Trefethen (p. 818) describe the U.K.-based e-Science program to design plug-and-play grid technologies for a range of disciplines. And Buetow (p. 822) outlines the ways in which cyberinfrastructure can weld together the vastly different styles of biomedical research.

For all the excitement, however, there are disturbing trends in the directions being taken by funding agencies that have historically been involved with driving the Internet revolution. In their Editorial (p. 757), Lazowska and Patterson consider how downsizing and short-term thinking threaten to derail the next generation of information innovation.

–DANIEL CLERY AND DAVID VOSS

CONTENTS

See also the Editorial on p. 757, News of the Week story by Daniel Clery, and related STKE material on p. 751 and at www.sciencemag.org/sciext/computers.

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DISTRIBUTED COMPUTING

NEWS

Grassroots Supercomputing

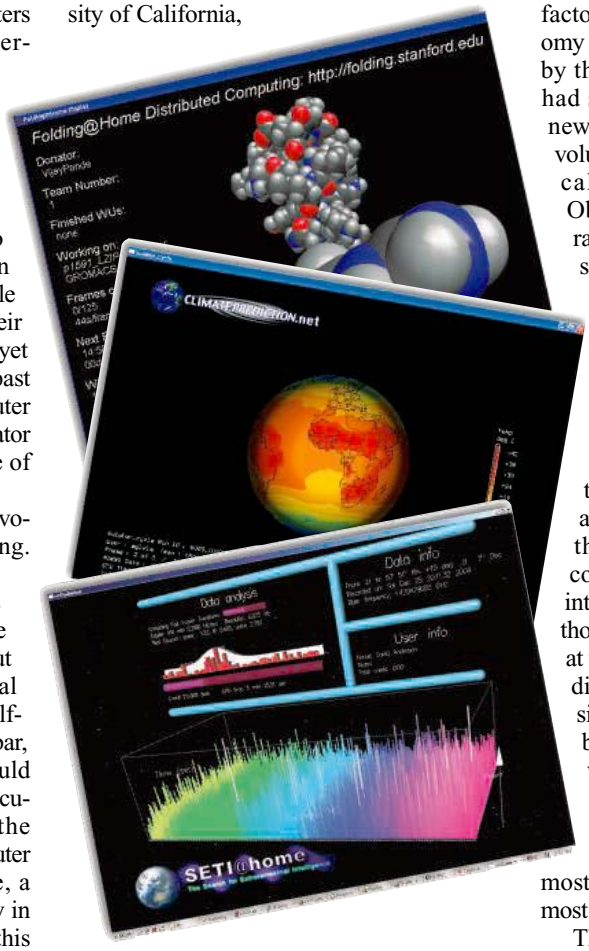
What started out as a way for SETI to plow through its piles of radio-signal data from deep space has turned into a powerful research tool as computer users across the globe donate their screen-saver time to projects as diverse as climate-change prediction, gravitational-wave searches, and protein folding

OXFORD, U.K.—If Myles Allen and David Stainforth had asked for a supercomputer to test their ideas about climate change, they would have been laughed at. In order to push the limits of currently accepted climate models, they wanted to simulate 45 years of global climate while tweaking 21 parameters at once. It would have required a supercomputer's fully dedicated attention over years, preempting the jealously guarded time slots doled out to many other projects. "Doing this kind of experiment wasn't even being considered," recalls Stainforth, a computer scientist here at Oxford University. So instead, he and Oxford statistician Allen turned to the Internet, where 100,000 people from 150 countries donated the use of their own computers—for free. Although not yet as flexible, their combined effort over the past 2 years created the equivalent of a computer about twice as powerful as the Earth Simulator supercomputer in Yokohama, Japan, one of the world's fastest.

Stainforth's project is part of a quiet revolution under way in scientific computing. With data sets and models growing ever larger and more complex, supercomputers are looking less super. But since the late 1990s, researchers have been reaching out to the public to help them tackle colossal computing problems. And through the selfless interest of millions of people (see sidebar, p. 812), it's working. "There simply would not be any other way to perform these calculations, even if we were given all of the National Science Foundation's supercomputer centers combined," says Vijay Pande, a chemical biologist at Stanford University in Palo Alto, California. The first fruits of this revolution are just starting to appear.

World supercomputer

Strangely enough, the mass participation of the public in scientific computing began with a project that some scientists believe will never achieve its goal. In 1994, inspired by the 25th anniversary of the moon landing, software designer David Gedye wondered "whether we would ever again see such a singular and positive event," in which people across the world join in wonder. Perhaps the only thing that could have that effect, thought Gedye, now based in Seattle, Washington, would be the discovery of extraterrestrial intelligence. And after teaming up with David Anderson, his former computer science professor at the University of California, Berkeley, and Woody Sullivan, a science historian at the University of Washington, Seattle, he had an idea how to work toward such an event: Call on the public to get involved with the ongoing Search for Extraterrestrial Intelligence (SETI) project.



Strength in numbers. Millions of computers now crunch data for diverse research projects.

In a nutshell, SETI enthusiasts argue that we have nothing to lose and everything to gain by scanning electromagnetic radiation such as radio waves—the most efficient method of interstellar communication we know of—from around the galaxy to see if anyone out there is broadcasting. After the idea for SETI was born in 1959, the limiting factor at first was convincing radio astronomy observatories to donate their help. But by the mid-1990s, several SETI projects had secured observing time, heralding a new problem: how to deal with the huge volume of data. One Berkeley SETI project, called SERENDIP, uses the Arecibo Observatory in Puerto Rico, the largest radio telescope in the world, to passively scan the sky around the clock, listening to 168 million radio frequencies at once. Analyzing this data would require full-time use of the Yokohama Earth Simulator, working at its top speed of 35 teraFLOPS (10^{12} calculations per second).

Gedye and his friends approached the director of SERENDIP, Berkeley astronomer Daniel Werthimer, and posed this idea: Instead of using one supercomputer, why not break the problem down into millions of small tasks and then solve those on a million small computers running at the same time? This approach, known as distributed computing, had been around since the early 1980s, but most efforts had been limited to a few hundred machines within a single university. Why not expand this to include the millions of personal computers (PCs) connected to the Internet? The average PC spends most of its time idle, and even when in use most of its computing power goes untapped.

The idea of exploiting spare capacity on PCs was not a new one. Fueled by friendly competition among hackers, as well as cash prizes from a computer security company, thousands of people were already using their PCs to help solve mathematical problems. A trailblazer among these efforts was GIMPS, the Great Internet Mersenne Prime Search, named after the 16th century French monk who discovered a special class of enormous numbers that take the form $2^P - 1$ (where P is a prime). GIMPS founder George Woltman, a programmer in Florida, and Scott Kurowski,

CREDITS (TOP TO BOTTOM): PHOTOS.COM; FOLDING@HOME; CLIMATEPREDICTION.NET; SETI@HOME

a programmer in California, automated the process and put a freely downloadable program on the Internet. The program allowed PCs to receive a task from the GIMPS server, "crunch" on it in the background, and send the results back without the PC user even noticing.

Using computer time in this way is not always a blameless activity. In 1999, system administrator David McOwen marshaled hundreds of computers at DeKalb Technical College in Clarkston, Georgia, to crunch prime numbers with a program from a distributed network—but without getting permission. When found out, he was arrested and accused of costing the college more than $400,000 in lost bandwidth time. But the case never came to court, and McOwen accepted penalties of 80 hours of community service and a $2100 fine. The previous year, computer consultant Aaron Blosser got the computers of an entire Colorado phone company busy with GIMPS. Because his supervisor had given him permission to do so, he was not charged, but because at the time it was considered a potential act of Internet terrorism, the FBI confiscated his computers.

Undaunted, Gedye and his team set about carving up the SETI processing work into bite-sized chunks, and in 1999 the team went public with a screen-saver program called SETI@home. As soon as a PC went idle, the program went to work on 100-second segments of Arecibo radio data automatically downloaded from the Internet, while the screen saver showed images of the signal analysis. It took off like wildfire. Within 1 month, SETI@home was running on 200,000 PCs. By 2001, it had spread to 1 million. Public-resource computing, as Anderson calls it, was born.

So far at least, SETI@home hasn't found an ET signal, admits Anderson, and the portion of the galaxy searched "is very, very limited." But the project has already accomplished a great deal: It not only fired up the public imagination, but it also inspired scientists in other fields to turn to the public for help tackling their own computing superproblems.

Democratizing science?

Stanford's Pande, who models how proteins fold, was among the first scientists to ride the public-resource computing wave. Proteins are like self-assembling puzzles for which we know all the pieces (the sequence of amino acids in the protein backbone) as well as the final picture (their shape when fully folded), but not what happens in between. It only takes microseconds for a typical protein to fold itself up, but figuring out how it does it is a computing nightmare. Simulating nanosecond slices of folding for a medium-sized protein requires an entire day of calculation on the fastest machines and years to finish the job. Breaking through what Pande calls "the microsecond barrier" would not only help us understand the physical chemistry of normal proteins, but it could also shed light on the many diseases caused by misfolding, such as Alzheimer's, Parkinson's, and Creutzfeldt-Jakob disease.

A year after SETI@home's debut, Pande's research group released a program called Folding@home. After developing new methods to break the problem down into workable chunks, they crossed their fingers, hoping that enough people would take part. For statistical robustness, identical models with slightly tweaked parameters were doled out in parallel to several different PCs at once, so success hinged on mass participation.

The simulations flooded back. By the end of its first year, Folding@home had run on 20,000 PCs, the equivalent of 5 million days of calculation. And the effort soon proved its worth. Pande's group used Folding@home to simulate how BBA5, a small protein, would fold into shape starting only from the protein's sequence and the laws of physics. A team led by Martin Gruebele, a biochemist at the University of Illinois, Urbana-Champaign, tested it by comparing with real BBA5. The results, reported in 2002 in *Nature*, showed that Folding@home got it right. This marks "the first time such a convergence between theory and experiment could be made," says Pande.

Public-resource computing now has the feel of a gold rush, with scientists of every stripe prospecting for the bonanza of idle computing time (see table, left). Biological projects dominate so far, with some offering screen savers to help study diseases from AIDS to cancer, or predict the distribution of species on Earth. But other fields are staking their own claims. Three observatories in the United States and Germany trying to detect the fleeting gravitational waves from cataclysmic events in space—a prediction of Einstein's—are doling out their data for public crunching through a screen saver called Einstein@home. Meanwhile, CERN, the European particle physics laboratory near Geneva, Switzerland, is tapping the public to help design a new particle accelerator, the Large Hadron Collider. LHC@home simulates the paths of particles whipping through its bowels.

Project/URL	Research Base	Goal
Mersenne Prime Search www.mersenne.org	Worldwide	Identify enormous prime numbers
SETI@home setiathome.ssl.berkeley.edu	UC Berkeley	Find extraterrestrial intelligence
Folding@home folding.stanford.edu	Stanford	Predict how proteins fold
ClimatePrediction.net climateprediction.net	Oxford	Test models of climate change
LHC@home lhcathome.cern.ch	CERN	Model particle orbits in accelerator
Einstein@home einstein.phys.uwm.edu	U.S. and Germany	Identify gravitational waves
Cancer Research Project www.grid.org/projects/cancer	NCI and Oxford	Search for candidate drugs against cancer
Lifemapper www.lifemapper.org	University of Kansas	Map global distribution of species

The projects launched so far have only scraped the surface of available capacity: Less than 1% of the roughly 300 million idle PCs connected to the Internet have been tapped. But there are limits to public-resource computing that make it impractical for some research. For a project to make good use of the free computing, says Stainforth, "it has to be sexy and crunchable." The first factor is important for attracting PC owners and persuading them to participate. But the second factor is "absolutely limiting," he says, because not all computational problems can be broken down into small tasks for thousands of independent PCs. "We may have been lucky to have chosen a model that can be run on a typical PC at all," Stainforth adds.

In spite of those limitations, the size and number of public-resource computing projects is growing rapidly. Much of this is thanks to software that Anderson developed and released last year, called Berkeley Open Infrastructure for Network Computing (BOINC). Rather than spending time and money developing their own software, researchers can now use BOINC as a universal template for handling the flow of data. In a single stroke, says Anderson, "this has slashed the cost of creating a public-resource computing project from several hundreds of thousands of dollars to a few tens of thousands." Plus, BOINC vastly improves the efficiency of the entire community by allowing PCs to serve several research projects at once: When one project needs a breather, another can swoop in rather than leaving the PC idle.

It works, too

As the data streams in from the many projects running simultaneously on this virtual supercomputer, some researchers are getting surprising results. To the initial dismay of CERN researchers, LHC@home occasionally produced very different outputs for the same model, depending on what kind of PC it ran on. But they soon discovered that it was caused by "an unexpected mathematical problem," says François Grey, a physicist at CERN: the lack of international standards for handling rounding errors in functions such as exponential and tangent. Although the differences between PCs were minuscule, they were amplified by the sensitive models of chaotic particle orbits. The glitch was fixed by incorporating new standards for such functions into the program.

The results of ClimatePrediction.net have been surprising for a different reason. "No one has found fault with the way our simulations were done," says Stainforth. Instead, climate scientists are shocked by the predictions. Reporting last January in *Nature*, a team led by Stainforth and Allen found versions of the currently accepted climate model that predict a much wider range of global warming than was thought. Rather than the consensus of a 1.5° to 4.5°C increase in response to a doubling of atmospheric CO_2, some simulations run on the Oxford screen saver predict an 11°C increase, which would be catastrophic. Critics argue that such warming is unrealistic because the paleoclimate record has never revealed anything so dramatic, even in response to the

Grid Sport: Competitive Crunching

You won't find the names of Jens Seitler, Honza Cholt, John Keck, or Chris Randles among the authors of scientific papers. Nor, for that matter, the names of any of the millions of other people involved with the colossal computing projects that are predicting climate change, simulating how proteins fold, and analyzing cosmic radio data. But without their uncredited help, these projects would be nonstarters.

In the 6 years since the SETI@home screen-saver program first appeared, scientists have launched dozens of Internet projects that rely on ordinary people's computers to crunch the data while they sit idle. The result is a virtual computer that dwarfs the top supercomputer in speed and memory by orders of magnitude. The price tag? Nothing. So who are these computer philanthropists? The majority seem to be people who hear about a particular project that piques their interest, download the software, and let it run out of a sense of altruism. Others may not even be aware they are doing it. "I help about a dozen friends with repairs and upgrades to their PCs," says Christian Diepold, an English literature student from Germany, "and I install the [screen-saver software] as a kind of payment. Sometimes they don't even know it's on there."

But roughly half of the data processing contributed to these science projects comes from an entirely different sort of volunteer. They call themselves "crunchers," and they get kicks from trying to churn through more data than anyone else. As soon as the projects began publishing data-crunching statistics, competition was inevitable. Teams and rank ladders formed, and per capita crunching has skyrocketed. "I'm addicted to the stats," admits Michael, a member of a cruncher team called Rebel Alliance. To get a sense of what makes them tick, *Science* interviewed dozens of crunchers in the Internet chat forums where they socialize.

Team players. Honza Cholt says crunchers have deep discussions about the science.

CREDITS: HONZA CHOLT

Interest in crunching does not appear to correlate strongly with background. For their day jobs, hard-core crunchers are parking lot attendants, chemical engineers, stay-at-home moms and dads, insurance consultants, and even, in at least one case, miners. Their distribution, like the Internet, is global. What's the motive? People crunch "for a diversity of reasons," says Randles, a British accountant who moderates the forum for ClimatePrediction.net, but altruism tops the list. "After losing six friends over the last 2 years to cancer, I jumped at the chance to help," says an electrician in Virginia who goes by the username JTWill and runs the Find-a-Drug program on his five PCs. As a systems administrator named Josh puts it, "Why let a computer sit idle and waste electricity when you could be contributing to a greater cause?"

But another driving force is the blatant competition. Michael of Rebel Alliance has recently built a computer from scratch for the sole purpose of full-time crunching, but he says he still can't keep up with Stephen, a systems engineer in Missouri and self-proclaimed "stats junkie" who crunches on 150 computers at once. Without the competition, "it wouldn't be as much fun," says Tim, a member of Team Anandtech who crunches for Folding@home. And like any sport, rivalries are soon simmering. "Members from different teams drop in on each other's forums and taunt each other a bit," says Andy Jones, a cruncher in Northern Ireland, "but it's all in good humor." As Anandtech team member Wiz puts it, "What we have here is community."

But where does this leave the science? Do crunchers care how the fruits of their labor are used, or do they leave it all to the researchers? It depends on the project, says Cholt, a sociology student in the Czech Republic, "but the communities that form often have long and deep discussions about the science." What holds the core of the crunching community together, says Seitler, a computer specialist in Germany, is the chance "for normal people to take part in a multitude of scientific projects." In some cases, crunchers have even challenged the researchers' published conclusions. "Many scientists would groan at the thought of nonscience graduates questioning their work," says Randles, but "scrutiny beyond peer review seems an important aspect to science."

Far from indifferent, crunchers can become virtual members of the research team, says François Grey, a physicist at CERN, the particle physics lab near Geneva, Switzerland, who helps run LHC@home. Above and beyond donating their computers, "they actually help us solve problems and debug software. And you have to keep them informed about what's going on with the project, or they get upset." Crunchers might not get credited on papers, says Grey, but "scientists have to treat this community with respect."

–J.B.

largest volcanic eruptions. Stainforth emphasizes that his method does not yet allow him to attach probabilities to the different outcomes. But the upshot, he says, is that "we can't say what level of atmospheric carbon dioxide is safe." The finding runs against recent efforts to do so by politicians.

And according to Stainforth, this illustrates something that makes public-resource computing a special asset to science. Rather than a hurdle to be overcome, "public participation is half of the goal." This is particularly true for a field like climate prediction, in which the public can influence the very system being studied, but it may also be true for less political topics. "We in the SETI community have always felt that we were doing the search not just for ourselves but on behalf of all people," says Sullivan. What better way to "democratize" science than to have a research group of several million people?

–JOHN BOHANNON

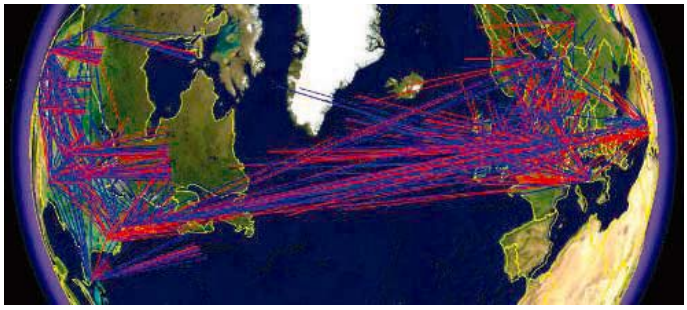
John Bohannon is a science writer based in Berlin.

NEWS

Data-Bots Chart the Internet

It's hard to map the global Internet from a small number of viewpoints. The solution may be to enlist computer users worldwide as local cartographers of cyberspace

Anyone who has tried to study the twists and turns in the data superhighway knows the problem: It is difficult even to get a decent map of the Internet. Because it grew up in a haphazard fashion with no structure imposed, no one knows how the myriad telephone lines and satellite links weave together its more than 300,000,000 computers. Today's best maps offer a badly distorted picture, incomplete and biased by a U.S. viewpoint, hampering computer scientists' efforts to design software that would make the Internet more stable and less prone to attack. But a new mapping effort may succeed where others have failed. "We want to let the Internet measure itself," says computer scientist Yuval Shavitt of Tel Aviv University in Israel, who, along with colleagues, hopes to enlist many thousands of volunteers worldwide to take part in the effort.



Gridlock. Accurate Internet maps could provide users with data traffic reports.

CREDIT: YUVAL SHAVITT AND ERAN SHIR/TEL AVIV UNIVERSITY

At the lowest level, the computers that comprise the Internet are known as "routers." They carry out the basic information housekeeping of the Net, shuttling e-mails and information packets to and fro. At a somewhat higher linked-facility level, however, the Internet can also be viewed as a network of subnetworks, or "autonomous systems," each of which corresponds to an Internet service provider or other collection of routers gathered together under a single administration. But how is this network of networks wired up?

Two years ago, computer scientist Kimberly Claffy and colleagues from the Cooperative Association for Internet Data Analysis at the University of California, San Diego, used a form of Internet "tomography" to find out. They sent out information-gathering packets from 25 computers to probe over 1 million different destinations in the Internet. Along the way, each packet recorded the links along which it moved, thereby tracing out a single path through the Internet—a chain of linked autonomous systems. Putting millions of such paths together, the researchers eventually built up a rough picture of more than 12,000 autonomous systems with more than 35,000 links between them (see www.caida.org/analysis/topology/as_core_network).

Through such efforts, researchers now understand that the Internet has a highly skewed structure, with some autonomous systems playing the role of organizing "hubs" that have far more links than most others. But researchers also know that their very best maps are still seriously incomplete.

The trouble is that all mapping efforts to date have started out from a fairly small number of sites, 50 at the most. So the maps produced tend to be biased by the locations of those sites. From some computer A, for example, researchers can send probing packets out toward computers B and C and thereby learn paths connecting A to B and A to C. But the probes would be unlikely to explore links between B and C, for the same reason that driving from New York to Boston and from New York to Montreal tells one little about the roads between Boston and Montreal. "If you send probes from only a few points, you naturally get a very partial point of view," says physicist Alessandro Vespignani, an expert on Internet topology at Indiana University, Bloomington.

To overcome this problem, Shavitt and colleagues are pioneering a new approach inspired by the idea of distributed computing. Anyone can now download a program from the Web site www.netdimes.org that will help in a global effort to map the Internet. Using no more than a few percent of the host computer's processing power, the program acts as a software agent, sending out probing packets to map local connections in and around the autonomous system in which the computer sits. "What we ask for is not so much processing power but location," says Shavitt. "We hope that the more places we have presence in, the more accurate our maps will be."

Since the project's inception late last year, individuals have downloaded nearly 800 agents that are now working together to map the Internet from 50 nations spread across all the continents. "We've already mapped out about 40,000 links between about 15,000 distinct autonomous systems, and we can already see that the Internet is about 25% denser than it was previously thought to be," says Shavitt. "This is a great project with a very new perspective," says Vespignani, who points out that better maps will help Internet administrators in predicting information bottlenecks and other hot spots.

Shavitt and his colleagues estimate that once they have about 2000 agents operating, it should be possible to get a complete map of the Internet at the autonomous-system level in less than 2 hours. Once they can do that, they hope to provide individual users with local Internet "weather reports." Ultimately, they would like to map the Internet at the level of individual routers—getting a more detailed map of the physical Internet. "We'll need about 20,000 agents distributed uniformly over the globe to get a good map at that level," says Shavitt. Then there'll be no excuse for getting lost in cyberspace.

–MARK BUCHANAN

Mark Buchanan is a writer in Cambridge, U.K.

Service-Oriented Science

Ian Foster

New information architectures enable new approaches to publishing and accessing valuable data and programs. So-called service-oriented architectures define standard interfaces and protocols that allow developers to encapsulate information tools as services that clients can access without knowledge of, or control over, their internal workings. Thus, tools formerly accessible only to the specialist can be made available to all; previously manual data-processing and analysis tasks can be automated by having services access services. Such service-oriented approaches to science are already being applied successfully, in some cases at substantial scales, but much more effort is required before these approaches are applied routinely across many disciplines. Grid technologies can accelerate the development and adoption of service-oriented science by enabling a separation of concerns between discipline-specific content and domain-independent software and hardware infrastructure.

Paul Erdös claimed that a mathematician is a machine for turning coffee into theorems. The scientist is arguably a machine for turning data into insight. However, advances in information technology are changing the way in which this role is fulfilled—by automating time-consuming activities and thus freeing the scientist to perform other tasks. In this Viewpoint, I discuss how service-oriented computing—technology that allows powerful information tools to be made available over the network, always on tap, and easy for scientists to use—may contribute to that evolution.

The practice of science has, of course, already been affected dramatically by information technology and, in particular, by the Internet. For example, the hundreds of gigabytes of genome sequence available online means that for a growing number of biologists, "data" is something that they find on the Web, not in the lab. Similarly, emerging "digital observatories" [already several hundred terabytes in dozens of archives (*1*)] allow astronomers to pose and answer in seconds questions that might previously have required years of observation. In fields such as cosmology and climate, supercomputer simulations have emerged as essential tools, themselves producing large data sets that, when published online, are of interest to many (*2*). An exploding number of sensors (*3*), the rapidly expanding computing and storage capabilities of federated Grids (*4*), and advances in optical networks (*5*) are accelerating these trends by making increasingly powerful capabilities available online.

Sometimes, however, the thrill of the Web seems to blind us to the true implications of these developments. Human access to online resources is certainly highly useful, putting a global library at our fingertips. But ultimately, it is automated access by software programs that will be truly revolutionary, simply because of the higher speeds at which programs can operate. In the time that a human user takes to locate one useful piece of information within a Web site, a program may access and integrate data from many sources and identify relationships that a human might never discover unaided. Two dramatic examples are systems that automatically integrate information from genome and protein sequence databases to infer metabolic pathways (*6*) and systems that search digital sky surveys to locate brown dwarfs (*7*).

The key to such success is uniformity of interface, so that programs can discover and access services without the need to write custom code for each specific data source, program, or sensor. Electric power–transmission standards and infrastructure enabled development of the electric power grid and spurred the development of a plethora of electric tools. In a similar manner, service technologies enable the development of a wide range of programs that integrate across multiple existing services for purposes such as metabolic pathway reconstruction, categorization of astronomical objects, and analysis of environmental data. If such programs are themselves made accessible as services, the result can be the creation of distributed networks of services, each constructed by a different individual or group, and each providing some original content and/or value-added product (*8*).

We see this evolution occurring in the commercial Internet. As the Web has expanded in scale, so the preferred means of finding things has evolved from Yahoo's manually assembled lists to Google's automatically computed indices. Now Google is making its indices accessible, spurring development of yet other services. What makes Google's indices feasible is the existence of large quantities of data in a uniform format (HTML, HyperText Markup Language) and—two important factors that must be considered when we turn to science—smart computer scientists to develop the algorithms and software required to manage the 100,000 computers used (at last count) to analyze Web link structure, and smart businesspeople to raise the money that pays for those computers!

The term "service-oriented architecture" refers to systems structured as networks of loosely coupled, communicating services (*9*). Thus, "service-oriented science" refers to scientific research enabled by distributed networks of interoperating services. [The term "e-Science," coined by John Taylor, has a similar but broader connotation (*10*).]

Creating and Sharing Services

Creating a service involves describing, in some conventional manner, the operations that the service supports; defining the protocol used to invoke those operations over the Internet; and operating a server to process incoming requests. A set of technologies called Web services (*9*) are gaining wide acceptance for these purposes. A variety of commercial and open-source Web services tools exist for developing services, deploying and operating services, and developing client applications. A fair amount of experience has been gained with the creation of services and applications in different science domains. Although problems remain (e.g., efficiency, interoperability of different vendor offerings), the technology is well beyond the experimental stage. Nevertheless, it can still be a big step to realize the full potential of service-oriented science, for reasons that I now discuss.

Interoperability. Services have little value if others cannot discover, access, and make sense of them. Yet, as Stein has observed (*11*), today's scientific communities too often resemble medieval Italy's collection of warring city states, each with its own legal system and dialect. Web services mechanisms for describing, discovering, accessing, and securing services provide a common alphabet, but a true lingua franca requires agreement on protocols, data formats, and ultimately semantics (*12*). For example, the definition of VOTable, a standard XML (eXtensible Markup Language)–based representation for tabular data (*13*), has been a powerful force for progress in astronomy.

Scale. Services must often deal with data volumes, computational demands, and numbers of users beyond the capacity of a typical PC. Responding to a user request—or to the arrival of new data—can involve large amounts of computation. For example, the Argonne GNARE system searches periodically through DNA and protein databases for new and updated genomes and then computes and pub-

Mathematics and Computer Science Division, Argonne National Laboratory, Argonne, IL 60439, USA, and Department of Computer Science, University of Chicago, Chicago, IL 60637, USA. E-mail: foster@mcs.anl.gov

lishes derived values (*14*) (Fig. 1). Analysis of a single bacterial genome of 4000 sequences by three bioinformatics tools (BLAST, PFAM, and BLOCKS) requires 12,000 steps, each taking on the order of 30 s of run time. GNARE is able to perform these tasks in a timely fashion only because it has access to distributed resources provided by two U.S. national-scale infrastructures, TeraGrid and Open Science Grid (see below).

The impact of automation on service load must also be considered. It is improbable that even a tiny fraction of the perhaps 500,000 biologists worldwide will decide to access GenBank, GNARE, or any other service at the same time. However, it is quite conceivable that 50,000 "agents" operating on their behalf would do so—and that each such agent would want to generate thousands of requests.

Management. In a networked world, any useful service will become overloaded. Thus, we need to control who uses services and for what purposes. Particularly valuable services may become community resources requiring coordinated management. Grid architectures and software—a set of Web services technologies focused on distributed system management—can play an important role in this regard (*15*).

Quality control. As the number and variety of services grow and interdependencies among services increase, it becomes important to automate previously manual quality-control processes—so that, for example, users can determine the provenance of a particular derived data product (*8*, *16*). The ability to associate metadata with data and services can be important, as can the ability to determine the identity of entities that assert metadata, so that consumers can make their own decisions concerning quality.

Incentives. A scientist may work long hours in the lab to obtain results that may bring tenure, fame, or fortune. The same time spent developing a service may not be so rewarded. We need to change incentives and enable specialization so that being a service developer is as honorable as being an experimentalist or theorist. Intellectual property issues must also be addressed so that people feel comfortable making data available freely. It is perhaps not surprising that astronomy has led the way in putting data online, given that its data have no known commercial value.

Scientists are certainly not alone in grappling with these challenges. However, science is perhaps unique in the scope and scale of its problems, the number and diversity of potential contributors, and the subtlety of the questions that service networks can be used to answer.

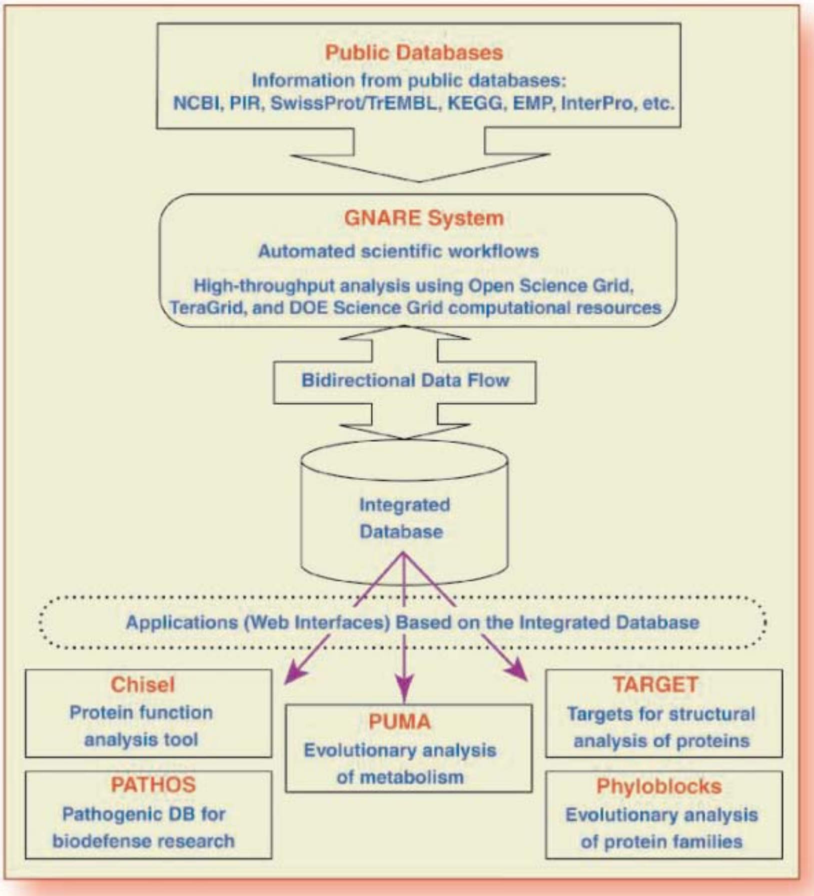


Fig. 1. What it can take to build a service. A powerful approach to the interpretation of newly sequenced genomes is comparative analysis against all annotated sequences in publicly available resources. Currently, the largest sequence database at the National Center for Biotechnology Information contains 2.3 million protein sequences. The precision of genetic sequence analysis and assignment of function to genes can be increased markedly by the use of multiple bioinformatics algorithms for data analysis. The GNARE system discussed in the text precomputes analysis results for every sequence, finding protein similarities (BLAST), protein family domains (BLOCKS), and structural characteristics. Grid resources are used to run the resulting millions of processes, a task that must be repeated frequently owing to the exponentially growing amount of data. [Image credit: Bioinformatics group, Mathematics and Computer Science Division, Argonne National Laboratory]

Rethinking Infrastructure

As scale increases, creating, operating, and even accessing services become increasingly challenging. How do we ensure that service-oriented science realizes its promise of being a democratizing force, rather than increasing the gap between the "haves" and "have-nots"?

Part of the solution is a familiar idea in commercial information technology, namely, outsourcing. Building and deploying a service require expertise and resources in three distinct areas: (i) the domain-specific content—data, software, and processes—that is to be shared; (ii) the domain-independent software functions needed to operate and manage the service and to enable community access, such as membership services, registries, metadata catalogs, and workflow orchestration services; and (iii) the physical resources—networks, storage, and computers—needed to host content and functions.

The last two capabilities—functions and resources—can, in principle, be handed off to specialist providers. If such specialists can deliver resources or operate required functions for many communities, then (again, in principle) economies of scale can be achieved, while scientists can focus on what they are good at—providing content and advancing science. In addition, individual services can scale more easily and efficiently when needed.

To see how this strategy can work, consider the SourceForge system, which provides hosting capabilities for communities developing open-source software. A new open-source project is provided with access to code archiving, mailing lists, and other related functions, as well as the hardware required to host those functions. This outsourcing of function and resource is made possible by the existence of the Internet infrastructure along with standard Web servers, browsers, and associated protocols, which together allow users (in this case, open-source communities) to focus on providing content (code) while SourceForge runs Web servers and related infrastructure.

In a similar manner, a "SourceForge for science" would both host scientific communities—operating community membership services, catalogs, storage services, work-

Fig. 2. The Open Science Grid links storage and computing resources at more than 30 sites across the United States to support a variety of services and applications, many concerned with large-scale data analysis. Circles show a subset of Open Science Grid sites; lines indicate communications, some with international partners. [Image credit: I. Legrand, Caltech]

flow orchestration services, and so forth—and provide access to the hardware resources required to operate both those functions and the application-specific services that constitute the communities "content." In this case, the supporting infrastructure must provide a much richer set of capabilities than does SourceForge, encompassing, for example, access control, accounting, provisioning, and related management issues. As noted above, Grid architectures and software (*15*) address many of these concerns, allowing users to focus on providing "content," which in this case comprises not just Web pages but also services, data, and programs.

SourceForge's hardware requirements are not substantial and thus can easily be provided by a centralized system. However, "cyberinfrastructure" (*17*) to support scientific communities need not be centralized. For example, the Open Science Grid (OSG) collaboration has constructed a distributed "Grid" linking clusters at 30 sites across the United States that total thousands of computers and tens of terabytes of storage (*18*) (Fig. 2). The Enabling Grids for eScience in Europe project, EGEE, has a similar structure. Major research universities and national laboratories participate in OSG and EGEE, but so do smaller institutions, which can thus enhance educational and research opportunities. For example, Florida International University is an important OSG resource provider, thanks to its 92-processor Linux cluster. All participants can obtain access to large quantities of distributed storage and computational power when they need it. These systems are being used by researchers in high-energy physics, biology, chemistry, radiology, and computer science.

This separation of concerns also suggests new roles for campus information technology organizations. In addition to operating commodity services such as Internet and e-mail, these organizations can host functions and provide resources.

Approaches to Scaling

The many groups working to apply service-oriented techniques to science are each exploring one or more of three different approaches to the problem of scaling. In the first, "cookie-cutter" approach, researchers create dedicated domain-specific infrastructures, in which uniformity is enforced across the board, at the content, function, and resource level. Here, the community standardizes the domain-specific software—and often also the hardware—that participants must deploy in order to provide required functions and resources. I give three examples of such systems.

The Biomedical Informatics Research Network, BIRN (*19*), is a National Institutes of Health initiative to facilitate collaboration in the biomedical sciences. BIRN has deployed standard compute and storage clusters at 19 sites across the United States. These systems, plus various functions such as catalogs and ontologies, support a variety of collaborative research programs in areas such as mouse brain morphology (*20*).

The National Science Foundation's Network for Earthquake Engineering Simulation, NEES, is a national collaboratory enabling community access to specialized instrument, data, and simulation resources for earthquake engineering. Each of its 17 instrument sites runs a NEES Point of Presence (a modest PC with a standard hardware configuration) with standard software enabling teleobservation, teleoperation, data collection, and related functions. Central services include catalogs and data archives. NEES has already enabled unique distributed experiments involving facilities at multiple sites (*21*).

The PlanetLab computer science testbed is a collection of several hundred PCs at universities and research laboratories worldwide, each with a standard configuration and each running standard software (*22*). Computer scientists can obtain access to "slices" on distributed collections of these PCs, on which they can deploy and evaluate experimental distributed services.

Pushing the electric power grid analogy perhaps farther than we should, cookie-cutter approaches give each participant their own electricity generator. This strategy has the advantage of achieving a high degree of central control and thus uniformity. On the other hand, the cost of expanding capability is high, requiring the acquisition and deployment of new hardware.

In the second, more bottom-up approach, researchers develop service ecologies in which agreements on interfaces allow participants to provide content and function in any way they see fit.

I referred above to the international virtual observatory community's VOTable format and to work in bioinformatics. The Department of Energy's Earth System Grid, ESG (*2*), is another example of a discipline-specific service that emphasizes the definition and implementation of standard interfaces. Building on the widely used OPeNDAP protocol for publishing and accessing environmental data, ESG has deployed services that provide access to over 100 TB of climate simulation data from the National Center for Atmospheric Research's Community Climate Simulation Model and other models involved in the International Panel on Climate Change assessment. Many terabytes of data are downloaded from these services each month.

As a second example, the UK myGrid project (*8*) has developed tools that allow biologists to define workflows that integrate information from multiple sources, including both biological databases and bioinformatics applications. These workflows can be archived and then run periodically to identify new phenomena of interest as, for example, in a recent study of Williams-Beuren syndrome (*23*).

For a third example, the Department of Energy's Fusion Collaboratory (*24*) operates services that enable online access to simulation codes. By reducing barriers to use, these services are increasing use of advanced computational techniques. Project members have also demonstrated on-demand coupling of simulation capabilities with physical experiments.

Continuing the electric power grid analogy, such service ecologies define relevant standards but leave each site to acquire and configure its own equipment. This approach has the advantage that the cost of entry can be low, particularly if appropriate software is available. On the other hand, individual service providers have no immediate means of scaling capability beyond acquiring more hardware.

The third approach involves the definition and deployment of general-purpose infrastructures that deliver discipline-independent resources or functions. I have already mentioned OSG and EGEE. As a third example, the National Science Foundation's TeraGrid links resources at nine sites across the United States, with each site deploying a common software distribution that permits secure remote access to computers and storage systems, monitoring of system components, accounting for usage, and so on. TeraGrid targets not only high-end "power users" but also the larger community through the deployment of "science gateways," discipline-specific services hosted on TeraGrid in support of specific communities.

General-purpose infrastructures can be compared with power plants, which operate to provide electricity to any consumer connected to the electric power grid. Like power plants, they have the potential to achieve economies of scale but also must grapple with the challenges of supporting many users with diverse requirements.

In addition to these national or transnational efforts, many university campuses are deploying "campus Grids" to support faculty and students. For example, Purdue University's NanoHub provides students and faculty with access to various applications, while the UCLA Grid federates multiple clusters across campus and provides online access to popular simulation codes.

These projects, and many others like them, are important experiments in the policies, organizational structures, and mechanisms required to realize service-oriented science. Elements of all three approaches will be required if we are to achieve broad adoption. In particular, it cannot be efficient for every scientist and community to become a service provider. Instead, individual communities—especially smaller communities—should be able to outsource selected functions and physical resources, thus allowing them to focus on developing their domain-specific content. The successful creation and operation of the service providers that support this outsourcing require both Grid infrastructure software and organizational and funding structures that expose real costs so that "build versus buy" decisions can be made in an informed manner.

Summary

Service-oriented science has the potential to increase individual and collective scientific productivity by making powerful information tools available to all, and thus enabling the widespread automation of data analysis and computation. Ultimately, we can imagine a future in which a community's shared understanding is no longer documented exclusively in the scientific literature but is documented also in the various databases and programs that represent—and automatically maintain and evolve—a collective knowledge base.

Service-oriented science is also a new way of doing business, with implications for all aspects of the scientific enterprise. Students and researchers must acquire new skills to build and use services. New cyberinfrastructure is required to host services, especially as demand increases. Policies governing access to services must evolve. Above all, much hard work must be done in both disciplinary science and information technology in order to develop the understanding needed for this potential to be fully exploited.

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VIEWPOINT

Cyberinfrastructure for e-Science

Tony Hey and Anne E. Trefethen

Here we describe the requirements of an e-Infrastructure to enable faster, better, and different scientific research capabilities. We use two application exemplars taken from the United Kingdom's e-Science Programme to illustrate these requirements and make the case for a service-oriented infrastructure. We provide a brief overview of the UK "plug-and-play composable services" vision and the role of semantics in such an e-Infrastructure.

It is no coincidence that it was at CERN, the particle physics accelerator laboratory in Geneva, that Tim Berners-Lee invented the World Wide Web. Given the distributed nature of the multi-institute collaborations required for modern particle physics experiments, researchers desperately needed a tool for exchanging information. After a slow start, the community enthusiastically adopted the Web for information exchange within their global experimental collaborations. Since its beginnings in the early 1990s, the Web has become an indispensable tool not just for the scientific world, but for the humanities, business, and recreation. Now, just a decade later, scientists are attempting to develop capabilities for collaboration that go far beyond those of the Web. Besides being able to access information from different sites, they want to be able to integrate, federate, and analyze infor-

Engineering and Physical Sciences Research Council, Polaris House, North Star Avenue, Swindon SN2 1ET, UK.

mation from many disparate and distributed data sources (including data archives as well as networks of sensors and identification tags) and to access and control computing resources and experimental equipment at remote sites. Such an infrastructure is in fact very close to the vision of linking computers and accessing remote data that J. C. R. Licklider took with him to the Defense Advanced Research Projects Agency, which initiated the research project that led to the ARPANET (which later became the Internet) (*1*).

One of the key drivers behind the search for such new scientific tools is the imminent deluge of data from new generations of scientific experiments and surveys (*2*). In order to exploit and explore the petabytes of scientific data that will arise from these high-throughput experiments, supercomputer simulations, sensor networks, and satellite surveys, scientists will need assistance from specialized search engines, data mining tools, and data visualization tools that make it easy to ask questions and understand answers. To create such tools, the data will need to be annotated with relevant "metadata" giving information as to provenance, content, conditions, and so on; and, in many instances, the sheer volume of data will dictate that this process be automated. Scientists will create vast distributed digital repositories of scientific data requiring management services similar to those of more conventional digital libraries, as well as other data-specific services. The ability to search, access, move, manipulate, and mine such data will be a central requirement for this new generation of collaborative science software applications.

In the United Kingdom, this vision was articulated by John Taylor, then director general of Research Councils at the Office of Science and Technology (OST)—a position roughly equivalent to that of the director of the National Science Foundation (NSF) in the United States. Taylor came from Hewlett-Packard, which has long had a vision of utility computing in which users in the future would be able to pay for information technology (IT) services as they required them, in the same way as we use conventional utilities such as electricity, gas, and water, or in pay-as-you-go telephone billing, rather than purchase IT infrastructure outright. Taylor recognized the trends in scientific collaboration summarized above and realized that many areas of science could benefit from a common IT infrastructure to support multidisciplinary and distributed collaborations. He therefore put together a successful bid to the UK government (*3*), and in 2001 the United Kingdom initiated a £250 million, 5-year e-Science program to develop the tools, technologies, and infrastructure to support such multidisciplinary and collaborative science. It is important to emphasize that e-Science is not a new scientific discipline; rather, the e-Science infrastructure developed by the program should allow scientists to do faster, better, or different research. This claim is best illustrated by two examples.

Two e-Science Exemplars

The global particle physics community is now planning a series of experiments to find the hitherto elusive Higgs boson. This particle is a key component of the successful Standard Model of Glashow, Salam, and Weinberg that is believed to unify the weak and electromagnetic interactions (*4*). At the CERN laboratory in Geneva, the world's most powerful particle accelerator—the Large Hadron Collider (LHC)—is under construction and is scheduled to be operational by 2007. However, finding experimental evidence for the existence of the Higgs particle will be a major technological challenge, because the characteristic signals of the Higgs are expected to be very rare and subtle. Experiments at the LHC will be on a scale greater than any other previous physics experiments, and each will generate several petabytes of data per year. The major experiments are collaborations of over 1000 physicists from over 100 institutions in Europe, America, and Asia. The experimental data, although initially generated at CERN, are distributed to groups of scientists all over the world. Not all of the analysis can be done in Geneva. Thus, very large amounts of data will need to be routinely distributed for subsequent analysis by teams of physicists at the collaborating institutions. In addition to the large volumes of experimental data, the particle physicists in each experiment will also create large samples of simulated data in order to understand the detailed behavior of the experimental detectors. The e-Science infrastructure required for these LHC experiments goes far beyond the capability to access data on static Web sites. The experimental particle physicists are therefore building a global infrastructure—the LHC Computing Grid—that will permit the transport and data mining of huge distributed data sets (*5*). This "middleware" infrastructure (so called because the software lies between the network and the application) will enable physicists to set up appropriate data sharing/replication/management services and to facilitate decentralized computational simulations and analysis.

A second and perhaps more typical example of multidisciplinary collaborative science is in the emerging field of systems biology. The UK program has recently funded a major e-Science project on Integrative Biology (*6*). This is a £2.3 million project led by Oxford University, whose goal is to develop a virtual laboratory for research on heart disease and cancer. The project involves four other UK universities, together with the University of Auckland in New Zealand. Denis Noble's group at Oxford are world-renowned for their research into models of the electrical behavior of heart cells. Peter Hunter and his team in the bioengineering department at the University of Auckland in New Zealand are doing pioneering research into mechanical models of the beating heart. Both groups are currently doing world-class research in their own specialist areas. However, the project aims to connect researchers in these two groups in a scientific virtual organization (VO). This VO is an environment that will allow researchers in the project (and only researchers in the project) to routinely access the models and data developed at both Oxford and Auckland, as well as allowing them access to computing resources and UK supercomputers. Of course, researchers have long been able to access resources at a remote site; here the intent is to put in place a comprehensive infrastructure that can provide users with a single sign-on capability that authenticates each user and authorizes access to specific resources at each site, automatically negotiating problems with firewalls and multiple administrative authorities. By providing a powerful and usable e-Science research environment in which these two groups can combine their research activities, it will be possible to investigate links between specific gene defects that affect the electrical behavior of heart cells and life-threatening heart arrhythmias. This is a type of research that neither group can do independently; it is in this sense that e-Science technologies can enable different science.

Cyberinfrastructure, e-Infrastructure, and the Grid

The high-speed national research networks that constitute the underlying fabric of the academic Internet have long connected scientific collaborations such as these. But now under the banner of e-Science, scientists and computer scientists around the world are collaborating to construct a set of software tools and services to be deployed on top of these physical networks. The goal is a core set of middleware services that will allow scientists to set up secure, controlled environments for collaborative sharing of distributed resources for their research. Collectively, these middleware services and the global high-speed research networks will constitute the new Cyberinfrastructure (in the United States) or e-Infrastructure (in Europe) for collaborative scientific research.

The term "Grid" was first used in the mid-1990s to denote a distributed computing infrastructure for advanced science and engineering. At that time, the idea was driven by a desire to use distributed computing resources as a metacomputer, and the name was taken from the electricity power grid, with the analogy that computing power would be made available for anyone anywhere to use. The Grid was a product of developing technologies in high-performance computers and networking, together with the 1980s Grand Challenges research program in the United States. In 2001,

Ian Foster, Carl Kesselman, and Steve Tuecke recognized the broader relevance of the Grid and redefined the Grid in terms of infrastructure to facilitate collaboration (*7*).

Unfortunately, present-day versions of Grid middleware provide only a small part of the functionality required for e-Science collaborations. Nevertheless, the vision of a set of middleware services that will allow scientists to set up VOs tailored to the needs of their specific e-Science communities has proved to have universal appeal. This vision is at the heart of the UK's e-Science program (*8*) and a similar vision is embodied in the Atkins report on Cyberinfrastructure for NSF (*9*).

Web Service Grids

Web Services are the distributed computing technology that the IT industry is uniting around to be the building blocks for interoperable, distributed IT systems (*10*). By encapsulating internal resources within the service and providing a layer of application logic between those resources and the consumers, the owners of the service are free to evolve its internal structure over time (for example, to improve its performance or dependability), without making changes in the message exchange patterns used by existing service consumers. This encourages loose coupling between consumers and service providers, which is important for building robust inter-enterprise IT systems, because no one party is in complete control of all parts of the distributed application.

Web Services have largely been developed to build VOs in the private sector. Most of the Web Services standards are being done in the context of the World Wide Web Consortium (W3C). The scientific community has been extending Web Services for scientific applications in the context of the Global Grid Forum (GGF). It is developing an Open Grid Services Architecture (OGSA) based on Web Services (*11*, *12*). By leveraging developments in Web Services technologies, e-Science application developers will be able to exploit the tools, documentation, educational materials, and experience from the Web Services community when building their applications. The e-Science community can focus on building the higher-level services specific to the application domain, while responsibility for the design of the basic components of a reliable underlying infrastructure is left to the IT industry. The GGF will soon publish standards and protocols for information services, execution management, data access and integration, resource management, and security. These basic services together with standards for portal technology and visualization services will enable scientists to use generic middleware infrastructure services to build their application-specific VOs. This is the rationale for the UK e-Science "plug-and-play composable services" vision for Grid middleware.

e-Science and Semantics

The UK e-Science program has around 100 projects covering many areas of science, engineering, and medicine. In areas such as astronomy and earth science, global communities are coming together to define common standards for data and metadata to allow sharing and access to information (*13*, *14*). Other scientists are using high-performance simulations, computational steering, and remote visualization to advance the state of the art in their respective fields (*15*, *16*). In engineering, companies such as Rolls Royce and BAESystems are exploring how such e-Science technology can assist them in exploiting new distributed applications (*17*, *18*). In bioinformatics, researchers and pharmaceutical companies are attempting to use e-Science technologies to reduce data to information and information to knowledge (*19*, *20*). And in medical informatics, there are ambitious projects on digital mammography and electronic patient records (*21*, *22*). Rather than enumerate such examples in detail, we shall look at two projects that are attempting to combine conventional data and computing technologies with technologies from the Semantic Web community (*23*).

The myGrid e-Science project is researching high-level middleware to support personalized in silico experiments in biology (*19*). These in silico experiments use databases and computational analysis rather than laboratory investigations to test hypotheses. In myGrid, the emphasis is on data-intensive experiments that combine the use of applications and database queries. These bioinformatics experiments often involve many processes or services that need to be orchestrated. Workflow tools enable this orchestration and help the biologist to design, describe, and record complex experiments in terms with which they can interact and that can also interact with the workflows of other researchers. Intermediate workflows and data are kept, notes and thoughts recorded, and different experiments linked together to form a network of evidence, as is currently done in bench laboratory notebooks.

The computer scientists and biologists in the project have together developed a detailed set of scenarios for investigation of the genetics of Graves' disease, an immune disorder causing hyperthyroidism, and of Williams-Beuren syndrome, a gene deletion disorder that affects multiple human systems and also causes mental retardation. To implement its ideas, the project has built a prototype electronic workbench based on Web Services. They have identified four categories of service: (i) external third-party services, such as databases, computational analyses, and simulations, wrapped as Web Services; (ii) services for forming and executing experiments, such as workflows, information management, and distributed database query processing; (iii) services for supporting the e-Science methodology, such as provenance and notification; (iv) semantic services, such as service registries, ontologies, and ontology management, that enable the user to discover services and workflows and to manage several different types of metadata. Some or all of these services are then used to support applications and build application services.

The project has developed a suite of ontologies (roughly speaking, agreed-on vocabularies of terms or concepts) to represent metadata associated with the different middleware services. Semantic Web technologies such as DAML+OIL (*24*) and standards body W3C's Web ontology language OWL (*25*) then allow the prototype myGrid workbench to reason over these services intelligently. The project has demonstrated the potential of such an approach for in silico bioinformatics experiments and is now attempting to produce more robust semantic components that will allow users to personalize their own research environments (*26*–*28*).

Another such project, Comb*e*Chem, has the ambitious goal of creating a Smart Laboratory for chemistry, using technologies for automation, semantics, and Grid computing (*29*–*31*). A key driver of the project is the fact that large volumes of new chemical data are being created by new high-throughput technologies, such as combinatorial chemistry, in which large numbers of new chemical compounds are synthesized simultaneously. The need for assistance in organizing, annotating, and searching this data is becoming acute. The multidisciplinary Comb*e*Chem team have therefore developed a prototype Smart Laboratory test bed that integrates chemical structure and property data resources with a Grid-based computing environment. The project has explored automated procedures for finding similarities in solid-state crystal structures across families of compounds and has evaluated new statistical design concepts to improve the efficiency of combinatorial experiments in the search for new enzymes and pharmaceutical salts for improved drug delivery. One of the key concepts of the Comb*e*Chem project is Publication@Source, though which there is a complete end-to-end connection between the results obtained at the laboratory bench and the final published analyses (*32*). In a sister project called eBank, raw crystallographic data are annotated with metadata and published by being archived in the UK National Data Store as a crystallographic e-print (*33*). Publications can then be linked back to the raw data for other researchers to access. The project has a vision for what they call a scholarly cycle, encompassing experimentation, analysis, publication, research, and learning (Fig. 1).

In another strand, computer scientists in the SmartTea project have worked with the Comb*e*Chem team to develop an innovative

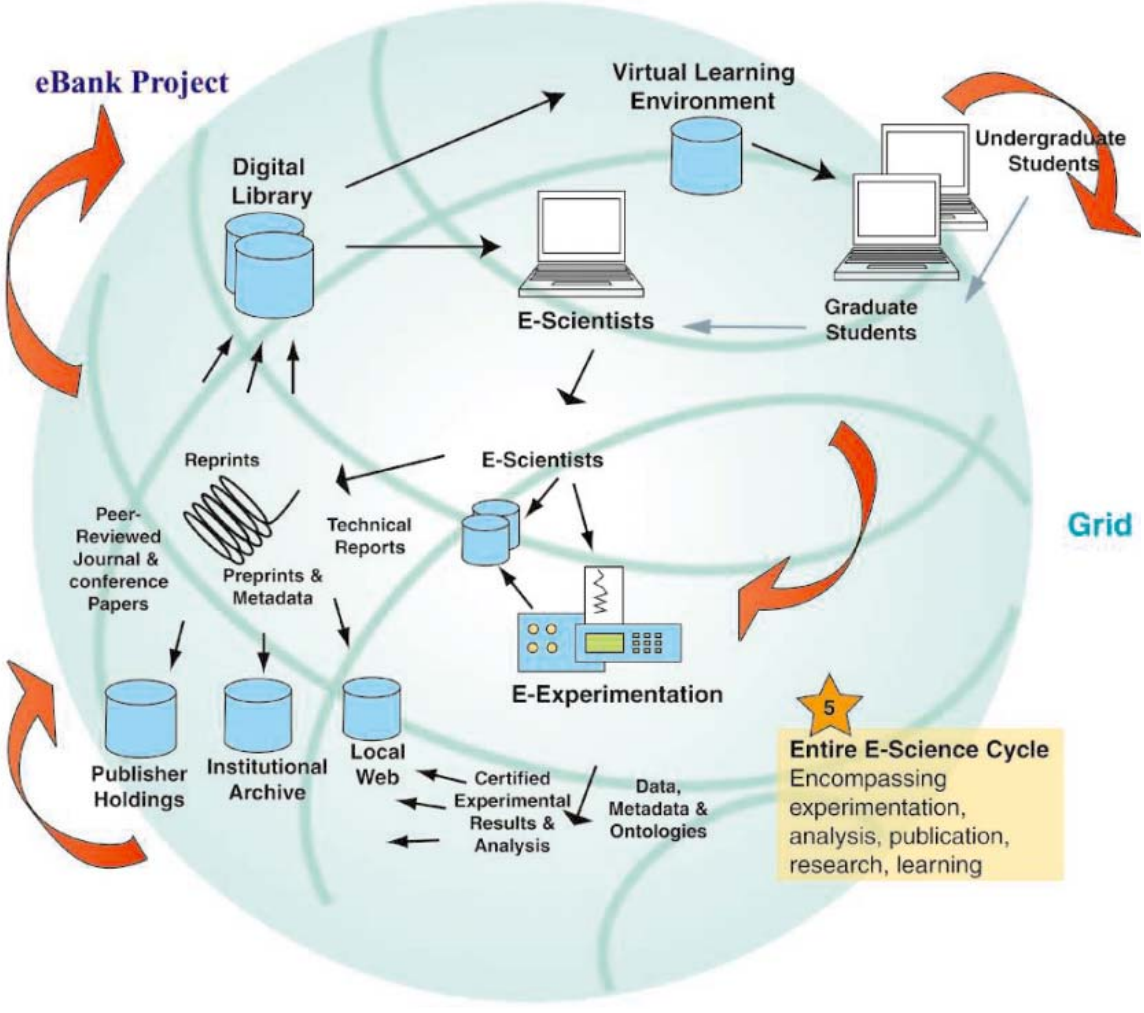

Fig. 1. The UK eBank project is focused on the changing landscape of scholarly communication, building links from e-research to e-learning, facilitating the scholarly knowledge cycle through the integration of digital repositories (experimental data, e-prints, and learning objects), and providing aggregator services. [Image courtesy of Liz Lyon and the eBank team]

human-centered system that captures the process of a chemistry experiment from plan to execution (*34*, *35*). They have used an analysis of the process of making tea in a laboratory to develop an electronic lab book replacement. Using tablet PCs, the system has undergone a successful trial in a synthetic organic chemistry laboratory and is linked to a flexible back-end storage system. A key finding was that users needed to feel in control, and this necessitated a high degree of flexibility in the lab book/user interface. The computer scientists on the team investigated the representation and storage of human-scale experiment metadata and introduced an ontology to describe the record of an experiment and a novel storage system for the data from the electronic lab book. In the same way in which the interfaces needed to be flexible to cope with whatever chemists wished to record, the back-end solutions needed to be flexible to store any metadata that might be created. Their storage system was based on Semantic Web technologies such as RDF (Resource Description Framework) and Web Services. This system was found to give a much higher degree of flexibility to the type of metadata that can be stored, as compared to traditional relational databases.

Toward a Semantic Grid

In 2001, De Roure, Jennings, and Shadbolt introduced the notion of the Semantic Grid, which advocated "the application of Semantic Web technologies both on and in the Grid" (*36*). From the requirements derived from the diverse set of UK e-Science applications, they identified a need for maximum reuse of software, services, information, and knowledge. Although the basic Grid middleware was originally conceived for hiding the heterogeneity of distributed computing, the authors contended that users now required "interoperability across time as well as space" to cope with both anticipated and unanticipated reuse of services, information, and knowledge. In a new paper, the same authors have revisited the e-Science program 3 years on from their original analysis to examine whether their expectations have been realized (*37*). They now see the e-Science requirements as a spectrum, with one end characterized by automation, virtual organizations of services, and the digital world, and the other end characterized by interaction, virtual organizations of people, and the physical world.

Conclusions

The broad view of Cyberinfrastructure/e-Infrastructure/Grid middleware services represented by the UK e-Science vision of plug-and-play composable middleware represents an exciting opportunity for both scientists and computer scientists. Although there is currently much focus in the Grid community on the low-level middleware, there are substantial research challenges for computer scientists to develop high-level intelligent middleware services that genuinely support the needs of scientists and allow them to routinely construct secure VOs and manage the veritable deluge of scientific data that will be generated in the next few years.

In the United Kingdom, we have therefore initiated a research program complementary to the e-Science application projects, whose goal is to explore the long-term computer science challenges arising from e-Science requirements (*38*). However, in parallel with this research thread, there is also the need to capture the prototype generic middleware services developed by our research projects and reengineer them for reuse by others. It is a major software engineering challenge to ensure that middleware components developed in the United Kingdom will interoperate with those developed in the United States, Asia, and elsewhere in Europe. This is the challenging mission for our newly established Open Middleware Infrastructure Institute (*39*).

In this article we have restricted our e-Science examples to those in the UK program (*40*). Needless to say, there are many other interesting e-Science projects in many countries of the world. Together, this global e-Science community is making progress toward realizing Licklider's vision for the Internet and in creating the components for a global middleware infrastructure. But there is still a long way to go before such middleware services can be used routinely by scientists going about their research.

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VIEWPOINT

Cyberinfrastructure: Empowering a "Third Way" in Biomedical Research

Kenneth H. Buetow

Biomedicine has experienced explosive growth, fueled in parts by the substantial increase of government support, continued development of the biotechnology industry, and the increasing adoption of molecular-based medicine. At its core, it is composed of fiercely independent, innovative, entrepreneurial individuals, organizations, and institutions. The field has developed unprecedented capacity to characterize biologic systems at their most fundamental levels with the use of tools and technologies almost unimaginable a generation ago. Biomedicine is at the precipice of unlocking the very essence of biologic life and enabling a new generation of medicine. Development and deployment of cyberinfrastructure may prove to be on the critical path to obtaining these goals.

The biomedical research community, dynamic and technology driven, shares its information through approaches initiated with Gutenberg's printing press and conceptually recognizable to scientists in the 18th century. Scientific findings are captured, summarized, and shared through manuscripts. The information infrastructure revolution that has transformed business and has had marked impact in other scientific disciplines has had slow uptake in biology and medicine.

Unquestionably, tremendous progress has been made in biomedicine through the application of information technology to this traditional information-sharing process. E-papers and e-journals and indices such as Pubmed all facilitate the sharing of manuscripts. Increasingly, biomedical journals require that primary data be deposited on a publisher's or investigator's Web-accessible site. In some communities, large centralized repository databases have been created for archiving biologic findings. These repositories support information retrieval through evolving current-art information technology [such as file transfer protocol (FTP) sites and Web browser portals]. For example, a recent plug-in for the Firefox Web browser permits researchers to have keyword access to these disparate data resources. However, like the communities that generate them, the infrastructure and information generated in biomedicine are largely disconnected and disjoint. Similarly, biomedical informatics, which I define as the application of information technology and its tools in biomedical disciplines (*1*), mirrors this structure of the culture it serves: highly heterogeneous in approach, small, independent, dispersed, and fragmented.

National Cancer Institute Center for Bioinformatics, National Institutes of Health, Rockville, MD 20892, USA. E-mail: buetowk@nih.gov

Biomedicine at a Crossroads

The current paradigms of information sharing and resource use in biology and medicine are being challenged on several fronts. First, the

success of the enterprise means that there has been a marked increase in the number of investigators, organizations, and institutions conducting biomedical research. Tracking the work and providing infrastructure to support the expansion are increasingly difficult. This expansion has resulted in a substantial number of new journals and Web sites. Although current information technology supports ready access, it does not address abstraction, integration, and interpretation of information. The diverse bioinformatics tools generated to consume and evaluate the data rarely interoperate. Commonly, the community demonstrates a willingness to share data and applications, but the number and diversity of components that must be assembled are overwhelming.

The very data generated in modern biomedicine presents a primary challenge to the researcher. Many of the new technologies used in today's research generate large volumes of rapidly expanding and ever-changing data. Although Moore's law and cheap disk space have reduced the impact of this growth, individual scientists and institutions are spending an increasing fraction of their effort and resources simply retrieving and processing data. Biologic data represents additional challenges. To integrate biologic data, one must traverse multiple orders of magnitude of scale and complexity. Ideally, in biology one would want to move seamlessly between biologic and chemical process, organelle, cell, organ, organ system, individual, family, community, and population. The diversity of data types that are explored in biomedicine is somewhat orthogonal. Technology permits the characterization of genomic, proteomic, metabolomic, image, and other large-scale characterizations.

All of the above is further confounded by the organization of biomedicine into research fields and disciplines. Such discipline focus generates an insidious challenge to information integration. Each community speaks its own scientific dialect. This community "speciation" results in reduced flow of information between disciplines, slowing the diffusion of knowledge and critical progress.

Finally, biomedicine's culture is at the nexus of a challenge faced by many other scientific fields: the need for "big" science and team science. The call for big science recognizes that many of the technology approaches required in biology and medicine are expensive, beyond the reach of individual investigators, and increasingly challenging the resource reserves of all but a few institutions. New paradigms are required to support these investigations. The push for team science also recognizes that many problems cross traditional discipline boundaries.

Cyberinfrastructure: A Third Way

A view that the current biomedical research culture is incompatible with team or big science is overly simplistic. It is clear that big science and team science will be necessary to achieve the goals of biology and medicine. However, the small, independent investigator is still the engine of innovative research. Widespread adoption of cyberinfrastructure represents an alternative in which the two approaches can be blended to create virtual team science. In so doing, the organization of biomedicine retains its entrepreneurial independent investigators whose insights and resources can be virtually joined through information technology. Big science contributes large-scale, raw material that feeds the virtual communities. Cyberinfrastructure empowers a reinvention of biomedicine without having to fundamentally change its basic culture or operational characteristics—a third way.

It is one thing to suggest that cyberinfrastructure could transform biomedicine and quite another thing to achieve this transformation. Fortunately, biomedicine can benefit from the long experience of other communities' embrace of informatics infrastructure to guide its approach. To address challenges in biomedicine, it must deliver in several key fronts. First, it must add perceivable value to the enterprise. In order to achieve widespread adoption, users must be motivated to do something different. Traditionally, this means they need to be able to do something they couldn't do without using the technology. Cyberinfrastructure shows great promise in this area because it has the ability to address the challenges of large, complex data sets. However, greater capacity may not be a sufficient driver, as demonstrated by current low penetration. Cyberinfrastructure will also need to enable new capabilities through the integration of communities and their disparate data types.

A primary lesson from other fields is that information technology has its greatest impact when it changes the way work can be performed. This may manifest itself through the apparent elimination of processing steps or the need to duplicate resources locally. Existing technologies permit the sharing and joining of common resources within virtual groups. However, the complex issues and diversity of biologic data still represent a substantial challenge to the creation of automated workflows.

Finally, the infrastructure needs to be easy to use and straightforward to implement. This requirement is more subtle than it might seem. A deeper examination raises the question, easy and straightforward to whom? Looking at the existing Internet and Web provides a useful clarification. End users consuming Internet resources through graphical user interfaces displayed through Web browsers would describe the Internet as easy to use. However, at the level of technical implementation, starting up a network that connects to the Internet and sharing information through a Web server is quite complex and beyond the skill set of an average biomedical researcher. It will be important to understand this dialectic as cyberinfrastructure is deployed across biology and medicine.

Biomedical research has experimented with the use of cyberinfrastructure to address the challenges outlined above for many years. An early example is found in the Cooperative Human Linkage Center (CHLC), a consortia formed early in the 1990s as part of the Human Genome Project for the purpose of creating genome-wide integrated genetic maps (*2*). CHLC was a geographically distributed virtual center connecting small specialized laboratories through informatics infrastructure communicating over the Internet (actually NSFnet at the time). It fulfilled a big-science need (creating the genetic map) through team science (each laboratory contributed specialized expertise) integrated virtually through current-art information technology. Each group worked in a context familiar to their specialized skills and the disparate parts were assembled by cyberinfrastructure to create the map. Map construction occurred through a pipelined workflow and used distributed processing over a network of multiuse computers. The raw data, analytic intermediates, and maps were distributed over the Internet through Web servers. The infrastructure to compute the maps was made available to the community through e-mail services. This example provides proof of concept that key aspects of the goals articulated above can be addressed, even with the use of a previous generation of information technology.

Technical Approach

The biology end user really doesn't care what technologies underlie cyberinfrastructure. Moreover, technology may not be the limiting factor in the development and deployment. However, the biomedical end user does provide key requirements that should be taken into consideration when choosing technology.

To facilitate adoption, cyberinfrastructure should be an extension of or interoperate with infrastructure already available to users. Ideally, it should integrate with and/or extend existing World Wide Web applications (supporting end-user needs) and Internet technology stacks (supporting the needs and existing investments of systems administrators where possible). Minimally, there must be a clear path from existing infrastructure to the new cyberinfrastructure.

The cyberinfrastructure vendor, operating system, and hardware should be as agnostic as possible. Users must have the capacity to change all of the above in order to maintain innovation and adjust to changing needs and developing technology. Open source is an oft-suggested solution to this. However, it can also be obtained by open standards and a commitment by those generating closed systems to adhere to these standards and to develop interfaces to communicate to and through them.

Biomedical cyberinfrastructure must also consider access and identity management as primary requirements. Although not unique to

biomedicine, protection of human subjects is required, as is the control and tracking of intellectual property and the need to establish academic credit and data provenance.

Many experiments are being implemented to explore alternative technologies that could possibly underlie cyberinfrastructure. These include peer-to-peer technology, Web services, and grid technology. Each has interesting potential. Grid technology has several distinguishing features (*3*, *4*). First, as a consequence of the widespread use of the Globus Toolkit (*5*) in various settings, grid technology is increasingly mature. Grid technology can support virtual communities through sharing of computational resources and data resources. Access and identity control are fundamental components of the architecture. The technology supports deterministic queries across a distributed, common schema. Its fundamental architecture also supports stateful processes important to the concept of workflow. The developing Open Grid Service Architecture–Data Access Integration (OGSA-DAI) framework holds promise for adding semantics to the grid technology so that computable, semantic interoperability may be achieved. Specific database schemas and data representations can be abstracted through a metadata layer. This information can be captured and shared in ontologies and services. This advance shows promise for machine capturing of information from the disparate biomedical communities and integrating of data and information into knowledge.

Grid architecture does have some key limitations. First, despite its developing research maturity, Grid is a distant second in commercial application. Web service architecture is the technology of choice for the vast majority of cyberinfrastructure support installations, in part because of the greater relative simplicity of the architecture. It is a straightforward extension of Internet and Web infrastructure familiar to the vast majority of systems designers and administrators. The broader developer and support base associated with Web services is important to the biomedical community.

Grid technology is not the only architecture with the capacity to address the challenges faced in biomedicine. However, what distinguishes Grid from, for example, Web services is that the capabilities described above are fundamental to the architecture. Web solutions to the challenges are outside the architecture and as such individually defined in each instance that they are created. The Grid architecture provides a standard framework for their representation and use. Encouragingly, Grid and Web services are converging.

Cyberinfrastructure in Action

As indicated above, the biomedical research community is conducting numerous experiments in developing and deploying cyberinfrastructure. With respect to Grid architecture, many are accessible through an index maintained by the Global Grid Forum (www.gridforum.org).

Many of these demonstration test beds explore the traditional definition of Grid computing in biomedicine, namely the sharing of resources across a virtual community. The range of these applications is impressive. They include molecular docking, protein structure determination, nucleic acid sequence alignment, and biologic feature extraction.

Several "proof-of-concept" test beds are exploring broad aspects of cyberinfrastructure in biomedicine, including the following:

Biomedical Informatics Research Network (BIRN). The BIRN project (www.nbirn.net) has focused on creation of geographically distributed virtual communities through shared resources. Its early work has been addressing the problems associated with new imaging platforms and the need to cross-correlate functional and structural data generated by these platforms. Its challenge is at the heart of cyberinfrastructure: How does one store, manage, curate, access, visualize, and analyze large volumes of data across a virtual community? Imaging projects generate terabytes of data through the use of disparate imaging technologies, all requiring compute-intensive applications to process.

BIRN has approached this problem by creating the virtual community through the distribution of a common, homogeneous, centrally configured hardware rack. This rack comes installed with appropriate software necessary to create the virtual community. The community is connected at high speed through the use of the Internet 2/Abiline backbone. It uses the Grid architecture defined by the Globus toolkit with numerous extensions, particularly in the areas of brokering storage resources across the community and the use of a metadata catalog.

A series of defined test beds are evaluating and extending the cyberinfrastructure, with a key focus of neuroimaging. Each test bed of defined members is exploring a dimension of the neuroimaging domain, with one centered around brain morphology, another around functional imaging (in schizophrenia), and the last around multiscale models in experimental systems (mouse).

myGrid. The myGrid project (www.mygrid.org.uk) takes a different perspective on application of cyberinfrastructure. Its focus is the support of investigator-driven experiments in silico. In myGrid, local and public data can be computationally evaluated to ask and answer questions in biology. It is less focused on resource sharing than BIRN, but rather strives to address issues related to semantic complexity of biologic data and the applications that process that data. It has constructed services that facilitate integration of data and applications. It addresses challenges associated with

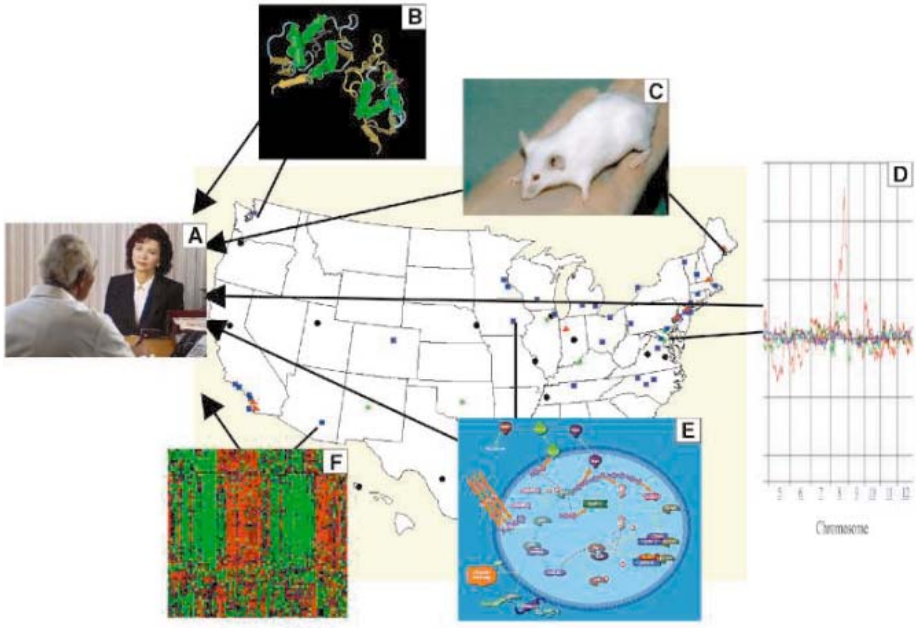

Fig. 1. The caBIG aims to integrate diverse biomedical research data so that investigators can consume data, services, and knowledge distributed throughout the research enterprise. For example, a scientist in California (**A**) designs an investigation following a computer modeling hypothesis-generating experiment where agent information from Washington (**B**) is queried in the context of animal model information from Maine (**C**). Genomic aspects of the experiment use comparative genome hybridization findings generated by colleagues in Maryland (**D**), which are interpreted in biologic processes from pathway data curated in Iowa (**E**). These are contrasted to reference expression signatures generated by researchers in Arizona (**F**).

the rapidly evolving nature of biomedical data and issue of data provenance. Particularly interesting is its approach to creating workflows. Within its framework it supports resource discovery and distributed queries.

myGrid is a service-based architecture whose core is Web services and OGSA-DAI. It uses the common Internet and does not require specialized hardware. It accomplishes its semantic interoperability through the use of ontology-based metadata. These metadata describe data, services, and other components of the infrastructure. The environment is open; however, it has the capacity to address the mixed data and service access requirements of researchers.

The myGrid project is exploring the diversity of the domains associated with biomedical cyberstructure. In one test bed, it has explored the circadian rhythms in *Drosophila melanogaster*. In a complementary test bed, it has supported genetic investigations of the human immune disorder Graves disease.

The cancer Biomedical Informatics Grid (caBIG). The approach of the caBIG project (http://caBIG.nci.nih.gov) to cyberinfrastructure is a conceptual hybrid between BIRN and myGrid. Similar to BIRN, its focus is to create a virtual community that shares resources and tackles the key issues of cyberinfrastructure. However, this community is open, spans the vast domain of cancer research, and is attempting to integrate the bench-to-bedside research cycle.

Similar to myGrid, it is an open infrastructure striving to achieve computational semantic interoperability. The caBIG's cyberinfrastructure is also a service-based architecture whose core is Web services and OGSA-DAI. It uses the common Internet and does not require specialized hardware. It has constructed services that facilitate integration of data and applications. Within its framework it supports resource discovery and distributed queries.

A key difference between myGrid and caBIG is the way they approach semantics and their related services. The caBIG cyberinfrastructure uses a common set of services and service registrations for the entire community. The shared caBIG semantic services provide biomedical ontologies and vocabularies in common use across biomedicine and cross-mappings between them. These mappings facilitate cross-disciplinary data integration and interpretation. The shared caBIG semantic services additionally include common data elements and object-based abstractions of the various research domains they serve. An open community process is used to maintain and extend these semantic resources. The use and registration of this common model-driven architecture serves as the basis of community-wide service descriptions. The caBIG test bed currently supports basic and translational research, clinical trials research, and tissue banking and pathology (Fig. 1). Participation in these groups is open.

Biomedical Cyberstructure and the Future

The above efforts suggest that it is technically feasible to knit the vibrant threads of biomedicine into a rich tapestry. There are still many challenges ahead, both technical and cultural. The differences indicate that there is not a single path joining biomedicine. As each effort reaches maturity it will be important to compare and contrast the lessons learned from their overlapping approaches. For example, how can the community ensure that existing individual, domain, and institution silos are not simply replaced with cybersilos?

Also, although these efforts are provocative, they have not yet crossed the threshold of demonstrated value. Evidence suggests that those in the field of biomedicine are receptive to exploring these alternatives but are still skeptical. Cyberinfrastructure appears to be up to the challenges confronting biomedical research. These are early but exciting times.

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Changes in Earth's Albedo Measured by Satellite

Bruce A. Wielicki,[1]* Takmeng Wong,[1] Norman Loeb,[2] Patrick Minnis,[1] Kory Priestley,[1] Robert Kandel[3]

The albedo of Earth, i.e., the fraction of the global incident solar radiation that is reflected back to space, is a fundamental parameter of global energy balance (*1*). Measurements from space since the 1970s give a global annual Earth albedo of ~0.29. The average incident solar radiative flux is 341 W m^{-2}, so that a change in albedo of 0.01 represents a global energy balance change of 3.4 W m^{-2}, similar in magnitude to the impact of doubling carbon dioxide in the atmosphere. Global albedo can change with changes in Earth's cloud fractional coverage, cloud thickness, aerosol amount, forest cover, or snow and ice cover. For example, a 2-year change in albedo was caused by the large Mount Pinatubo volcanic eruption in June 1991. Stratospheric aerosols from the eruption increased global albedo by up to 0.007 because of the reflection of an additional 2.5 W m^{-2} of solar radiation over the following 2 years (*2*, *3*). A recent report (*4*) claims to have detected an even larger increase in albedo, although not connected to any specific event like a volcanic eruption, between 2001 and 2003. We examined recent global satellite observations designed to measure the variations in planetary albedo, the broadband CERES (Clouds and the Earth's Radiant Energy System) observations from the NASA Terra spacecraft, in order to determine if substantial changes occurred over that period.

Figure 1 shows the anomalies in albedo from the monthly time series of the CERES global satellite measurements, which began in March 2000. To eliminate the large seasonal cycle in the data, we deseasonalized the CERES monthly anomalies by differencing each January from the average of all four January months from March 2000 through February 2004. The data plotted is from the CERES Terra FM1 instruments Edition 2 ES-4 data product (*5*, *6*). Anomalies are shown versus the 4-year average and are given in terms of global reflected broadband shortwave flux as well as in global albedo units. The CERES data cover the entire Earth, for the entire solar spectrum from 0.3- to 4-μm wavelength. The earthshine results are primarily for visible wavelengths and represent about half of Earth's surface (*4*).

The global CERES observations show a small decrease of ~2 W m^{-2} in shortwave reflected flux, equal to an albedo decrease of 0.006. These results stand in stark contrast to those of Pallé *et al.* (*4*), which show a large increase of 6 W m^{-2} or an albedo increase of 0.017, as shown for comparison in Fig. 1.

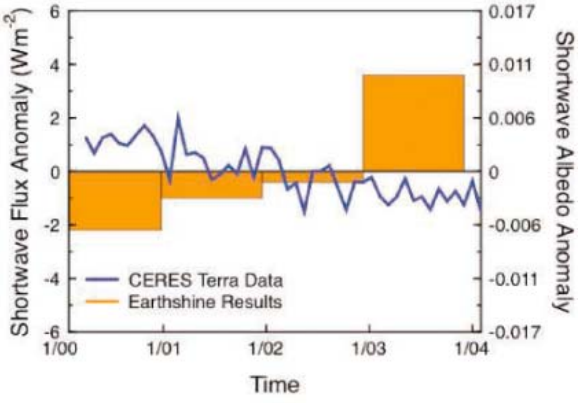

Fig. 1. Comparison of global satellite anomalies in reflected solar flux for 2000 through 2003. Earthshine results from (*4*) are shown in orange, and blue indicates the global satellite results from the CERES radiation budget instrument designed to measure global albedo from the NASA Terra spacecraft.

Comparison of independent observations from the two Terra CERES instruments indicate that ~1.1 W m^{-2} of the decrease in reflected flux observed by CERES FM1 may be explained by ultraviolet radiation exposure during a hemispheric scan mode used early in the observations. This would further reduce the CERES anomaly to 0.9 W m^{-2}. The FM1 instrument used this hemispheric scan mode for half of the first 2 years in orbit and has remained in normal cross-track Earth imaging since November 2001.

What is the effect of albedo change on climate? If the change is caused by changing land surface, aerosols, or snow and ice cover, then the earth should cool with increasing albedo and warm with decreasing albedo. This is because these changes in the Earth system have large effects on reflected solar radiation but much smaller effects on emitted thermal infrared cooling to space. If such changes had occurred at the magnitude of the earthshine data in (*4*), a global cooling twice the level of the ~0.25°C of the Pinatubo eruption would be expected, even over short time periods (*3*). Such a cooling has not been observed.

A second possibility would be a large decrease in global ocean heat storage. Observations of annual mean global ocean heat storage for 1992 through 2002 (*7*) show an 0.7 W m^{-2} increase in global ocean heat storage from 2000 to 2002. Sampling noise in the ocean heat flux is estimated at 0.4 W m^{-2} at 1σ (*7*). To be directly comparable to global reflected solar flux changes, the ocean heat storage flux was scaled from the ocean-only area used by Willis *et al.* (*7*) to global surface area. If only albedo changes were occurring, the ocean heat storage data would require an 0.7 ± 0.8 W m^{-2} decrease in reflected flux from 2000 to 2002, with 95% confidence. This change is consistent with the CERES data but not with the earthshine results.

The above discussion, however, considers only the effect of albedo change. Cloud changes would affect both albedo and Earth's thermal infrared cooling to space. Could this be the cause of changes in albedo that are not affecting surface temperature or ocean heat storage? We examined the CERES global thermal infrared radiative fluxes and the Moderate Resolution Imaging Spectrometer (MODIS) global derived cloud properties (CERES Edition 2 SSF data), but neither showed the large cloudiness changes that would be required to match an increased global albedo from 2000 to 2003 (*4*). The much smaller CERES flux and MODIS cloud changes are still within interannual variability, and a longer Terra data record is required to evaluate key issues like cloud feedback in the climate system.

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[1]NASA Langley Research Center, 21 Langley Boulevard, Hampton, VA 23681, USA. [2]Department of Atmospheric Science, Hampton University, Hampton, VA, USA. [3]Laboratoire de Meteorologie Dynamique du CNRS, Palaiseau, France.

*To whom correspondence should be addressed. E-mail: b.a.wielicki@nasa.gov

Functional Genomic Analysis of the Wnt-Wingless Signaling Pathway

Ramanuj DasGupta,[1]*† Ajamete Kaykas,[2]* Randall T. Moon,[2]† Norbert Perrimon[1]†

The Wnt-Wingless (Wg) pathway is one of a core set of evolutionarily conserved signaling pathways that regulates many aspects of metazoan development. Aberrant Wnt signaling has been linked to human disease. In the present study, we used a genomewide RNA interference (RNAi) screen in *Drosophila* cells to screen for regulators of the Wnt pathway. We identified 238 potential regulators, which include known pathway components, genes with functions not previously linked to this pathway, and genes with no previously assigned functions. Reciprocal-Best-Blast analyses reveal that 50% of the genes identified in the screen have human orthologs, of which ~18% are associated with human disease. Functional assays of selected genes from the cell-based screen in *Drosophila*, mammalian cells, and zebrafish embryos demonstrated that these genes have evolutionarily conserved functions in Wnt signaling. High-throughput RNAi screens in cultured cells, followed by functional analyses in model organisms, prove to be a rapid means of identifying regulators of signaling pathways implicated in development and disease.

Wnt proteins are a family of conserved signaling molecules involved in a plethora of fundamental developmental and cell biological processes such as cell proliferation, differentiation, and polarity (*1*–*3*). Several components of the pathway are tumorigenic when mutated in hepatic, colorectal, breast, and skin cancers (*1*, *4*, *5*). Wnts encode secreted glycoproteins that activate receptor-mediated pathways (*6*), which lead to numerous transcriptional and cellular responses. The main function of the Wnt–β-catenin pathway is to stabilize the cytoplasmic pool of a key mediator, β-catenin (β-cat) [called Armadillo (Arm) in *Drosophila*], which is otherwise degraded by the proteosome pathway. Initially identified as an important player in stabilizing cell-cell adherens junctions, β-cat is now known to participate in transcriptional regulation by forming a complex with the T cell–specific transcription factor (TCF) and lymphoid enhancer–binding factor (LEF) families of high-mobility-group (HMG)–box transcription factors (*7*, *8*). In cells stimulated by Wnts, stabilized β-cat translocates to the nucleus, where, together with TCF/LEF transcription factors, it activates downstream target genes (*7*, *8*). The Wnt pathway can also be activated through inhibition of its negative regulators such as glycogen synthase kinase-3β (GSK-3β), adenomatous polyposis coli (APC), and Axin, which promote degradation of β-cat, or by the introduction of activating mutations in β-cat that render it incapable of interacting with the degradation complex. Wnt signaling can also activate an alternative signaling pathway involved in planar cell polarity (PCP) that may lead to protein kinase C (PKC) and Jun kinase (JNK) activation, resulting in calcium release and cytoskeletal rearrangements (*9*, *10*).

Whole-genome RNA interference screens. Genetic and biochemical approaches have identified many of the genes that regulate the Wnt-Wg pathway in *Drosophila* (*11*) and other model organisms. However, many components may remain unidentified if mutants do not display a distinguishable "Wnt phenotype." Indeed, it is estimated that only 25% of all known *Drosophila* genes are associated with a readily obvious phenotype (*12*–*15*). The availability of the *Drosophila* genome sequence, a well-established RNA interference (RNAi)–based screening technology, and the fact that ~75% of the fly genome remains uncharacterized, provided us with an opportunity to rapidly and systematically characterize gene function at a genomewide scale to find new components in the Wnt signaling pathway (*16*–*19*).

Here we present the results from a genome-wide RNAi screen in *Drosophila* cells that identified 238 potential regulators of the Wnt pathway. These include many known genes that have not been implicated previously in the Wnt pathway, as well as others that have not yet been assigned any gene function. We further demonstrate the conserved involvement of selected candidate genes in the Wnt-Wg pathway by conducting functional assays in *Drosophila* and mammalian cells. Finally, these cell-based assays were complemented by analysis of the functions of selected genes at the organismic level, specifically in *Drosophila* and in the zebrafish embryo.

Wnt reporter genes and screen design. The assay for the RNAi screen was based on the Wnt reporter TOP-Flash (TCF optimal promoter), which consists of multimerized TCF-binding sites driving the expression of a cDNA encoding the firefly luciferase gene (*20*, *21*). The screen was performed in *Drosophila* imaginal disc–derived clone 8 cells, which are epithelial in origin (*22*, *23*). The Wg pathway is active in the imaginal discs, and thus clone 8 cells are likely to contain the majority of the components required to respond to Wg (*24*). The assay involved transfection of the TOP-Flash reporter, along with a *Renilla* luciferase vector (PolIII-RL) as a control for transfection efficiency, and an expression vector encoding *wg* (pMK33-wg) to stimulate the pathway (*24*–*27*) (see fig. S1). The activity of the Wg signaling pathway was quantified by measurement of normalized (N) luciferase expression or relative luciferase activity units (RLUs), which equated to the ratio of the absolute activity of firefly luciferase to that of renilla luciferase.

To optimize the Wg assay for a high-throughput screen (HTS) in a 384-well plate format, we designed two new TOP-Flash–like reporters, STF16 and dTF12, because existing reporters did not display robust signal-to-noise ratio in the high-density screen format (fig. S2) (*28*). STF16 comprises 16 TCF-binding sites and a minimal TATA box from the thymidine kinase promoter, whereas dTF12 contains 12 TCF-binding sites upstream of the *Drosophila* heat shock minimal promoter (fig. S2A). We first optimized the reporter assays in 96-well plate format (fig. S2, B and C). Although the reporters exhibited different basal activities, both allowed use of small volumes of cells and transfection reagents and displayed strong signal-to-noise ratios in multiple *Drosophila* cell lines including clone 8 and S2 receptor–positive ($S2R^+$) cells (*24*) (fig. S2B). Both reporters were expressed in a robust fashion after pathway stimulation by Wg, as well as by downstream activators in the pathway such as a ΔNLrp6, a constitutively active form of the Wg coreceptor low-density lipoprotein (LDL)

[1]Department of Genetics, Howard Hughes Medical Institute (HHMI), Harvard Medical School, New Research Building, No. 339, 77 Avenue Louis Pasteur, Boston, MA 02115, USA. [2]HHMI, Department of Pharmacology, Center for Developmental Biology, University of Washington, School of Medicine, Seattle, WA 98195, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: rdasgupt@genetics.med.harvard.edu (R.D.) or perrimon@receptor.med.harvard.edu (N.P.) and rtmoon@u.washington.edu (R.T.M.)

receptor–related protein-6 (LRP6) (*29*) (fig. S2B). The use of two independent reporters interchangeably in primary and secondary screens ensured robustness of the assay by minimizing any reporter-specific differences and/or artifacts. The specificity of the reporters was confirmed by the use of FOP-Flash (in which the 12 TCF-binding sites are mutated), which did not display any significant activity above background (fig. S2C).

RNAi-mediated knockdown of positive regulators, such as Arm and *Drosophila* TCF (dTCF), suppressed Wg-enhanced reporter activity, whereas RNAi-knockdown of negative regulators, such as Axin, ectopically activated the reporter in the absence of stimulus or further synergistically activated the reporter when induced by Wg or LRP6 (Fig. 1A; fig. S2). Thus, we could use this reporter to identify both positive and negative modulators (Fig. 1B).

Data analysis and validity of primary screen. For the whole-genome RNAi screen for the Wnt pathway (fig. S1) (*28*), we used a library of ~22,000 double-stranded RNAs (dsRNAs) (*30*). The library represents >95% of genes in the *Drosophila* genome and has been used successfully in several screens (*24*, *26*, *27*, *31*). The screen was performed in duplicate to reduce the rate of false-positives and to ensure the reproducibility of and hence confidence in individual candidate genes. To ascertain potential candidate genes involved in the Wnt pathway, we analyzed the data from each individual plate with four distinct protocols, and we assigned candidate genes on the basis of their deviation from the plate average for each given criterion [see Methods (*28*)]. Genes that satisfied two or more statistical criteria were considered strong candidates; those that scored positive only by one imposed condition were considered weak candidates.

We identified 238 candidates that showed consistent response in both screens that either reduced or increased Wnt pathway activity as measured by the TOP-Flash reporter activity (table S1, A and B). A majority of the known core Wnt pathway members were identified, including Wnt-*wingless* (*wg*) (*32*), *arrow* (*arr*)/LRP-6 (*33*), *frizzled* (*fz*) (*34*), *frizzled-4* (*fz4*), *dallylike protein* (*dlp*), *naked cuticle* (*nkd*) (*35*), *axin* (*axn*) (*36*), *supernumerary-limbs* (*slmb*) (*37*), *casein kinase 1 alpha* (*ck1α*), *disheveled* (*dsh*) (*38*), *β-catenin–armadillo* (*β-cat–arm*) (*39*), *dTCF/pangolin* (*dTCF/pan*) (*40*), the gene for *Drosophila* cAMP-responsive element–binding protein (CREB)-binding protein (*dCBP*)/*nejire* (*nej*) (*41*), *pygopus* (*pygo*) (*42*), and *legless* (*lgs*) (*43*), thus underscoring the robustness and validity of the Wnt screen in this HTS format (Fig. 2B). Comparison of the *z* scores (which measure the number of standard deviations away from the mean for any particular normalized luciferase value) between the duplicate screens revealed high reproducibility both qualitatively and quantitatively, with a correlation coefficient of 0.63 (Fig. 2A). Note that ~90% (213 out of 238) of the candidate genes that were selected for further analyses were verified in secondary screens (table S1A). About 50% of the genes identified in the screen had an associated Gene Ontology annotation or had an identifiable InterPro protein domain. Many of these genes corresponded to certain molecular complexes or biological functions, including (i) HMG- and homeodomain-box transcription factors, (ii) kinases and phosphatases, (iii) proteosomal components and ubiquitin ligases, (iv) small GTPases (guanosine triphosphatases: monomeric guanine nucleotide–binding proteins) family, (v) membrane-associated proteins, and (vi) cellular enzymes (Fig. 2C).

Among the 52 potential transcription factors identified in the screen, several contain HMG-box protein domains. In fact, the proteins of the TCF/LEF family that interact with β-cat in the nucleus to activate Wnt target genes themselves encode HMG transcription factors. Additionally, recent studies in *Xenopus* embryos have suggested that β-cat can physically interact with other HMG-box transcription factors, such as Sox family members, to regulate transcription of endodermal genes (*44*). Even though the specificity of these interactions in the Wnt pathway will have to be further tested, our results indicate that there may be other HMG transcription factors that cooperate with β-cat in the regulation of downstream Wnt target genes.

Several members of the TATA-binding protein (TBP)–associated factors (Taf) family of transcription factors were identified. There is evidence from both in vitro studies in mammalian cells and in vivo studies in *Drosophila* that β-cat physically interacts with TBP and that there are other cofactors such as Pontin and Reptin (Repressing Pontin) that interact with both TBP and β-cat to regulate Wnt target gene activity antagonistically (*45*, *46*). Intriguingly, dsRNA knockdown of most Tafs led to an increase in Wnt reporter activity, which suggests that Tafs might contribute to repression of Wnt target genes. Other classes of transcription factors identified in the screen include several homeodomain-containing and *HOX* genes. There is precedence for cross talk between Wnts and homeodomain or Hox transcription factors. HOXB13 inhibits TCF-4–mediated Wnt signaling activity in prostate cells by decreasing expression of Tcf-4 and its target genes (*47*). On the other hand, zebrafish *wnt8* transcriptionally regulates *vent* and *vox* genes encoding two homeodomain transcription factors in the establishment of the ventral pattern in the early embryo (*48*).

Protein phosphorylation and dephosphorylation by protein kinases and phosphatases have been especially implicated in the regulation of β-cat protein stability and degradation (*7*–*9*). Recent studies have also suggested that Wnt signaling stimulates and requires the phosphorylation of Lrp5 and 6–Arrow intracellular domain (PPPSP motif) to create an inducible docking site for Axin, a scaffolding protein controlling β-cat stability (*49*). We identified

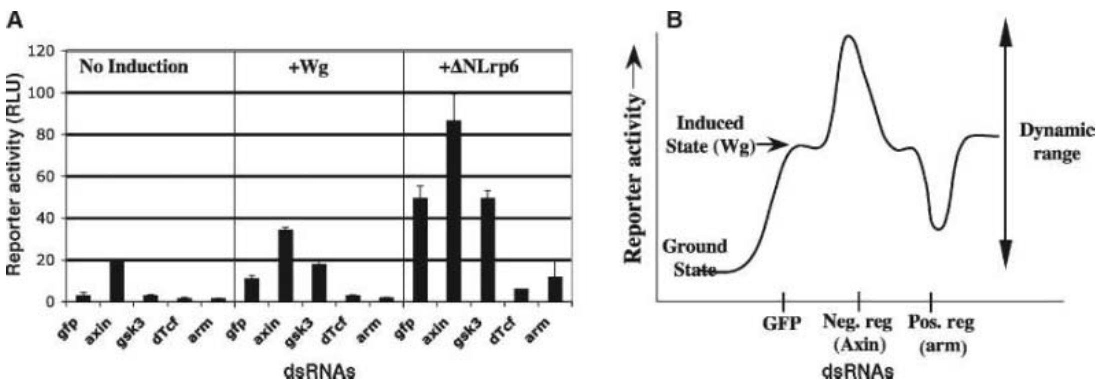


Fig. 1. Wg reporter assay. (**A**) Optimization of reporter assay in 384-well plate format with both Wg and ΔNLrp6 as activators. dsRNA knockdown of the known negative regulator, Axin, activates the reporter in uninduced cells, whereas knockdown of control positive regulators such as Arm and dTCF represses Wg-induced activation of the TOP-Flash reporter. Note the further activation of the Wg reporter upon dsRNA-mediated knockdown of Axin over and above Wg- or ΔNLrp6-mediated induction of reporter. Knockdown of Gsk3β did not affect reporter activity in clone 8 cells(1d) even though its knockdown resulted in activation of STF16 or dTF12 reporters in the absence of Wg induction, in S2R+ cells (fig. S2). (**B**) Schematic representation of 1A demonstrating the use of the reporter to screen for both positive and negative regulators in a single assay.

several protein kinases that negatively or positively affected the activity of the Wnt reporter gene. These include genes that encode known members of the pathway such as *ck1α*; genes whose function in the Wnt pathway has not been previously recognized—such as *warts* and *PDGF- and VEGF-receptor related* (*pvr*) [platelet-derived growth factor and vascular endothelial growth factor, respectively]; and genes encoding kinases that have no annotated function (see table S1A).

We also identified a class of proteins containing one or more Armadillo repeats (Arm repeats). The Arm-repeat protein motif was first identified in the *Drosophila arm* gene and is a tandemly repeated sequence motif about 40 amino acids long. Arm-repeat proteins function in various processes, such as intracellular signaling and cytoskeletal regulation, and include such proteins as β-cat, the junctional plaque protein plakoglobin, the APC tumor suppressor protein, and the nuclear transport factor importin-α. These repeats have a key role in mediating protein-protein interactions between β-cat and other important regulators of the Wnt pathway (*50*). A subset of these proteins is conserved across eukaryotic kingdoms. Taken together, our results indicate that there are likely to be additional Arm-repeat proteins that participate in the regulation of the Wnt pathway.

Additionally, we used "Reciprocal-Best-BLAST" (RBB) and other BLAST protocols to identify potential human homologs of the genes identified in the screen (for details, see table S2). These analyses indicated that >50% of the genes identified in the RNAi screen have vertebrate orthologs, which suggests their potential conserved role in the Wnt signaling pathway across evolution (see Fig. 2C and below for functional validation in mammalian cells). To test whether the genes identified in the screen were involved in the regulation of the Wnt pathway in multiple cell types, we performed the reporter assay for the selected candidate genes in multiple *Drosophila* cell lines including S2R$^+$ and Kc167 cells (table S3). Of the 200+ genes, we found ~140 genes that appear to regulate Wnt signaling activity in two or more cell types. Our analysis suggests that a majority of the candidate genes is not specific to clone 8 cells but is more generally required for the modulation of the Wnt signaling pathway in multiple cell types.

Secondary screens. A challenge presented by any high-throughput primary screen is to be able to extract meaningful information from the list of candidate genes. One useful approach is to categorize groups of genes according to their putative function in specific secondary assays that can be designed on the basis of previous knowledge of the signaling pathway. To accomplish that for the Wnt screen, we ordered the candidate genes in the Wnt pathway in an epistatic relation according to their roles at various steps in the pathway in relation to

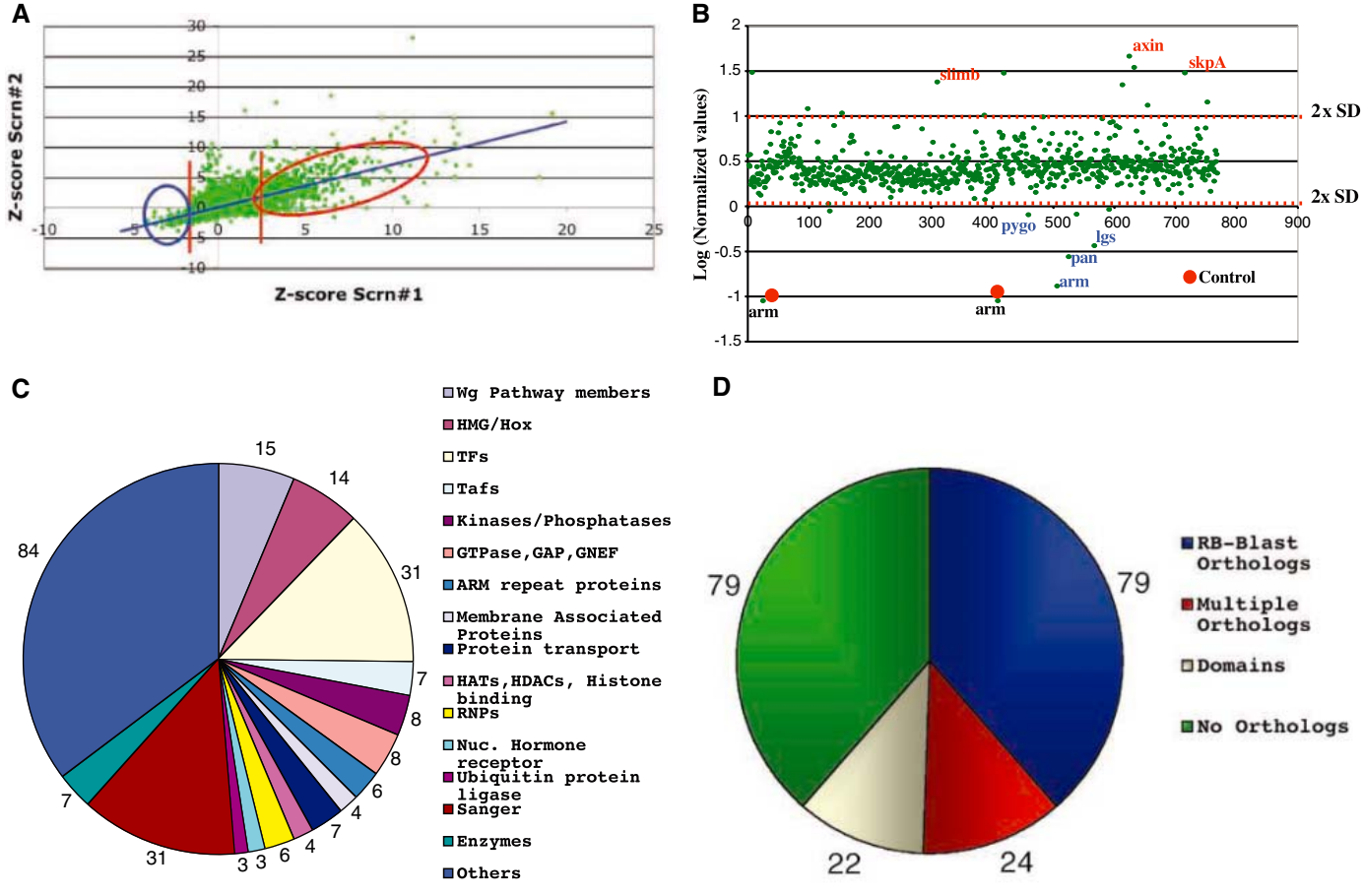


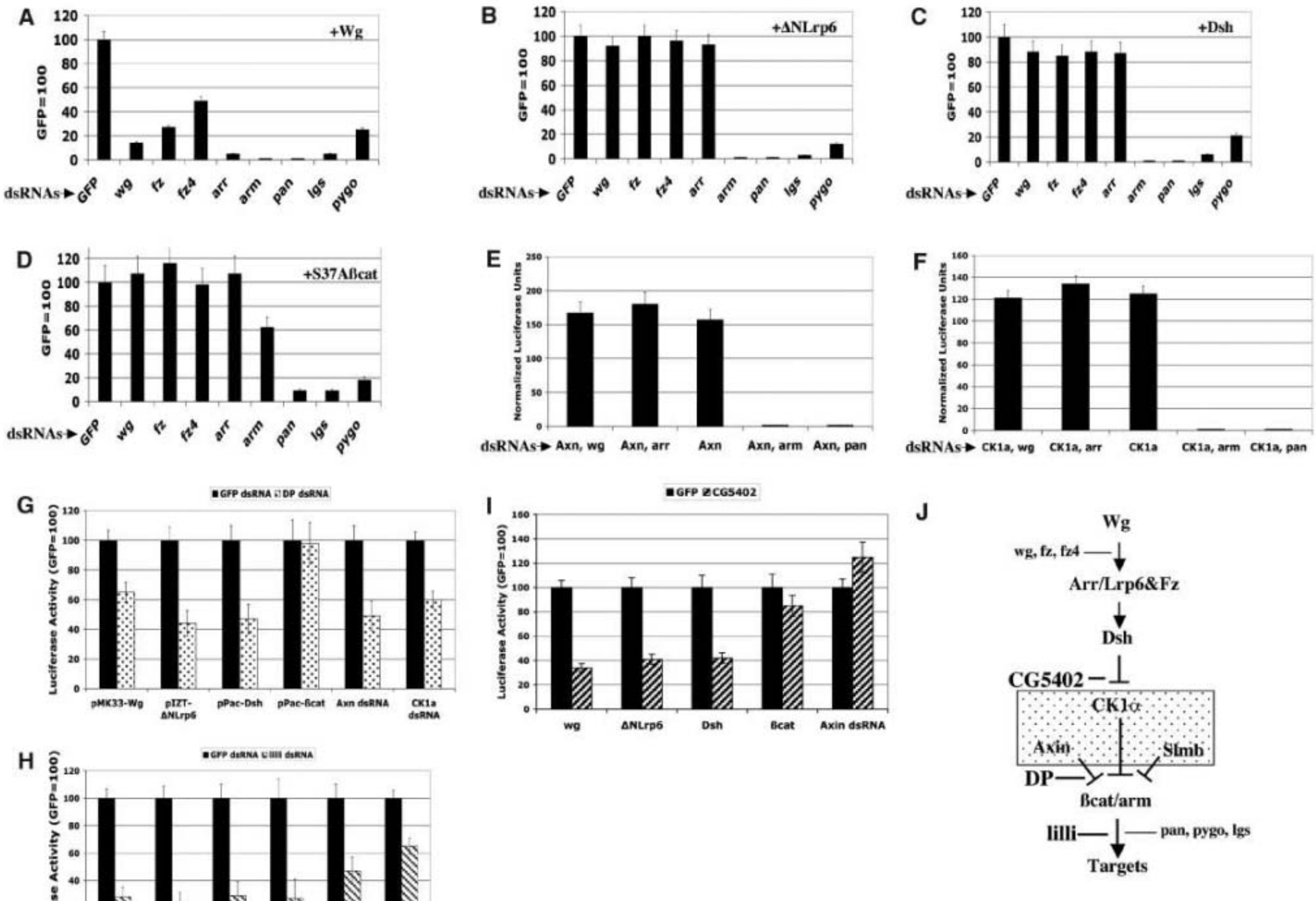
Fig. 2. Data analysis for the Wg screen. (**A**) Scatter plot comparison of *z* scores obtained from duplicate whole-genome screens, screen 1 versus screen 2. "Edge effect" outliers were removed. The comparison reveals a high correlation between the duplicate screens, with most data points mapping to a diagonal line (blue) in the scatter plot. The correlation coefficient between the two screens was 0.63. Data points within the blue oval were considered to be candidate genes that act as potential positive regulators of the Wg pathway in clone 8 cells, whereas the ones within the red oval were considered potential negative regulators. (**B**) Scatter plot of two representative plates that contained several of the known positive (blue) and negative (red) regulators of the pathway with respect to other data points and the controls from cells expressing arm dsRNA (red dots). (**C**) Candidate genes obtained from the primary screen as potential regulators of the Wg pathway based on their "Gene Ontology" and molecular function or protein domains. (**D**) The percentage of the total number of candidate genes obtained from the Wg screen that have potential vertebrate orthologs, as judged by Reciprocal Best Blast (details in table S2).

known negative and positive regulators of the pathway (Fig. 3).

To conduct epistasis experiments, we activated the signaling pathway either by transfecting individual DNA constructs encoding activators of the pathway [Wg, ΔNLrp6, Dsh, or β-cat (Fig. 3, A to D)] along with the reporter gene or by dsRNA-mediated inhibition of known negative regulators [Axin, CK1α (Fig. 3, E and F), or Slimb]. We used RNAi to knock down expression of individual candidate genes during simultaneous activation of the pathway by different inducers. We used both Wg reporters (fig. S1A) in our secondary screens for independent confirmation of our assays. Simultaneous expression of dsRNA for known downstream positive regulators, together with genes encoding activators of the pathway, inhibited reporter activation. For example, activation of the pathway by overexpression of Dsh was blocked by RNAi knockdown of genes encoding downstream effectors (*arm*, *pan*, *pygo*, or *lgs*) but not that of upstream pathway members encoding the ligand-receptor complex (*wg*, *arr*, *fz*, or *fz4*) (Fig. 3C). Alternatively, ectopic activation of the reporters that occurred after dsRNA-mediated knockdown of negative regulators (such as *axin*, or *ck1α*) could be efficiently inhibited by RNAi of downstream positive regulators (such as *arm* or *pan*) but not by dsRNAs directed toward components (such as *wg*, *arr*, or *fz*) that act upstream of *axin* and *ck1α* (Fig. 3, E and F).

These results allowed us tentatively to place selected candidate genes in a hierarchy either upstream or downstream of known positive and negative regulators. Specific examples of three potential regulators that we identified in the screen (Fig. 3, G to I) include two known transcription factors, DP (dimerization partner) and Lilli (Lilliputian), and a novel gene, *CG5402*, as activators in the Wnt pathway in the primary screen. In vitro epistasis experiments in clone 8 cells placed each of the three candidate genes at three distinct steps in the pathway (Fig. 3, G to I). CG5402 acts upstream of Axin but downstream of Wg, Fz, or Arr (Fig. 3I); DP functions downstream of Axin and Ck1α but upstream of β-cat (Fig. 3G); and Lilli functions downstream of β-cat (Fig. 3H). It is interesting that *lilli* encodes an HMG-box transcription factor. *lilli* has also been shown to interact genetically with *arm*, which further corroborates its role in the Wnt pathway (*51*). It is important to note that *lilli* interacts genetically with members of several signaling pathways, including the receptor tyrosine kinase (RTK)/Ras and the Decapentaplegic (Dpp) pathway, which underscores the power of the RNAi approach in assigning functions to genes with pleiotropic functions that may be critical factors involved in cross talk between multiple signaling pathways (*52*, *53*).

Overall, our epistasis analysis of the potential positive regulators in the clone 8 cells failed to place any new gene between Wg-Fz-Arr ligand-receptor complex and Dsh (*54*), even though known intermediates such as Arr and



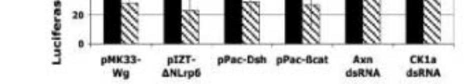


Fig. 3. Epistasis analysis of selected candidate genes in clone 8 cells. (**A** to **F**) Reporter gene activity in cells expressing indicated dsRNAs and various activators or inducers of the pathway such as coexpression of positive regulators, for example, Wg (A), ΔNLrp6 (B), Dsh (C), or S37Aβ-cat cDNA (D); or dsRNA-mediated knockdown of negative regulators such as Axin (E) or Ck1α (F). Relative luciferase activity units (RLUs) with reporter activity of cells containing GFP dsRNA and the inducer scaled to 100 units. (**G** to **I**) Effect of dsRNA-mediated knockdown of three selected candidate genes on TOP-Flash reporter activity in clone 8 cells, including DP transcription factor, Lilli, and CG5402, after induction of the pathway. (**J**) Epistatic ordering of the selected candidate genes in G to I and positive controls in A to F.

Fz were placed between Wg and Dsh by this method (Fig. 3, A to D and J). Preliminary epistasis analysis of most genes encoding potential positive regulators revealed that they affect the pathway downstream of Dsh. These genes were further categorized into those that acted upstream or downstream of genes involved in phosphorylation or degradation of β-cat (*axin*, *ck1α*, and *slmb*) and those that acted downstream of β-cat (*54*). Altogether, the in vitro epistasis studies provide a starting point from which to investigate the mechanism of action of candidate genes identified in the screen.

The candidate genes that increased reporter activity when their expression was inhibited were further tested in order to categorize them into specific functional groups. First, we determined whether RNAi of potential negative regulators could ectopically activate the TOP-Flash reporter in the absence of Wg stimulus. Of the 129 negative regulators tested, 63% (83 out of 129) activated reporter activity after dsRNA-mediated knockdown, which suggests a potential role in the regulation of basal Wg activity in a cell (table S4). Genes in this category could be either directly or indirectly acting at the level of regulation of Arm/β-cat stability and/or phosphorylation or at the level of target gene regulation. RNAi knockdown of the remaining 47 genes promoted expression of the TOP-Flash reporter only in the presence of Wg, which suggests a role specifically in Wg-stimulated cells. This second class of genes could be functioning either at the level of ligand-receptor regulation or receptor-mediated endocytosis, or they may be involved in the regulation of the stable pool Arm/β-cat that is present only in a stimulated cell. This class includes regulators, such as *nkd* and *Dlp*, that have been shown to regulate the intracellular and extracellular trafficking of Wg, respectively (*55*–*57*).

We tested whether decreased expression of ''candidate'' negative regulators required downstream effectors such as Arm and Pan to activate the Wnt–β-cat–responsive reporter gene (fig. S3). We transfected cells with *arm* or *pan* dsRNA together with individual dsRNAs specific for selected negative regulators. With the exception of two genes, *CR31616* and *CG4699*, Arm and Pan were indeed required for activation of the TOP-Flash reporter (in the absence of Wg stimulus), which placed them epistatically downstream of most negative regulators (fig. S3).

In vivo validation of hits in *Drosophila*. To further test the relevance of the genes identified as potential regulators of the Wnt pathway, we overexpressed selected candidates in cells in culture and in *Drosophila* wing imaginal discs in vivo (Fig. 4). One of the candidate genes encoded the small GTPase Rab5 (*58*, *59*). Rab5 has a central role in early endocytic trafficking by directing the budding of endocytic vesicles from the plasma membrane, their movement along microtubules, and their fusion with sorting endosomes. Rab5 has been implicated in controlling the shape of the long-range gradient of the transforming growth factor superfamily member, Dpp, in the *Drosophila* wing by regulating the endocytosis of ligand-receptor complex (*60*). Rab5-interacting proteins, such as APPL1 and APPL2, as well as other proteins involved in the formation of clathrin-coated vesicles (CCVs) (such as Eps15, epsin, and β-arrestin 2), can undergo nucleocytoplasmic shuttling and can interact with nuclear transcription factors to regulate expression of target genes (*61*, *62*). These studies indicate that the endocytic machinery may be directly involved in nuclear signaling functions as well (*62*).

In our screen, RNAi-mediated depletion of Rab5 only promoted reporter activity if cells

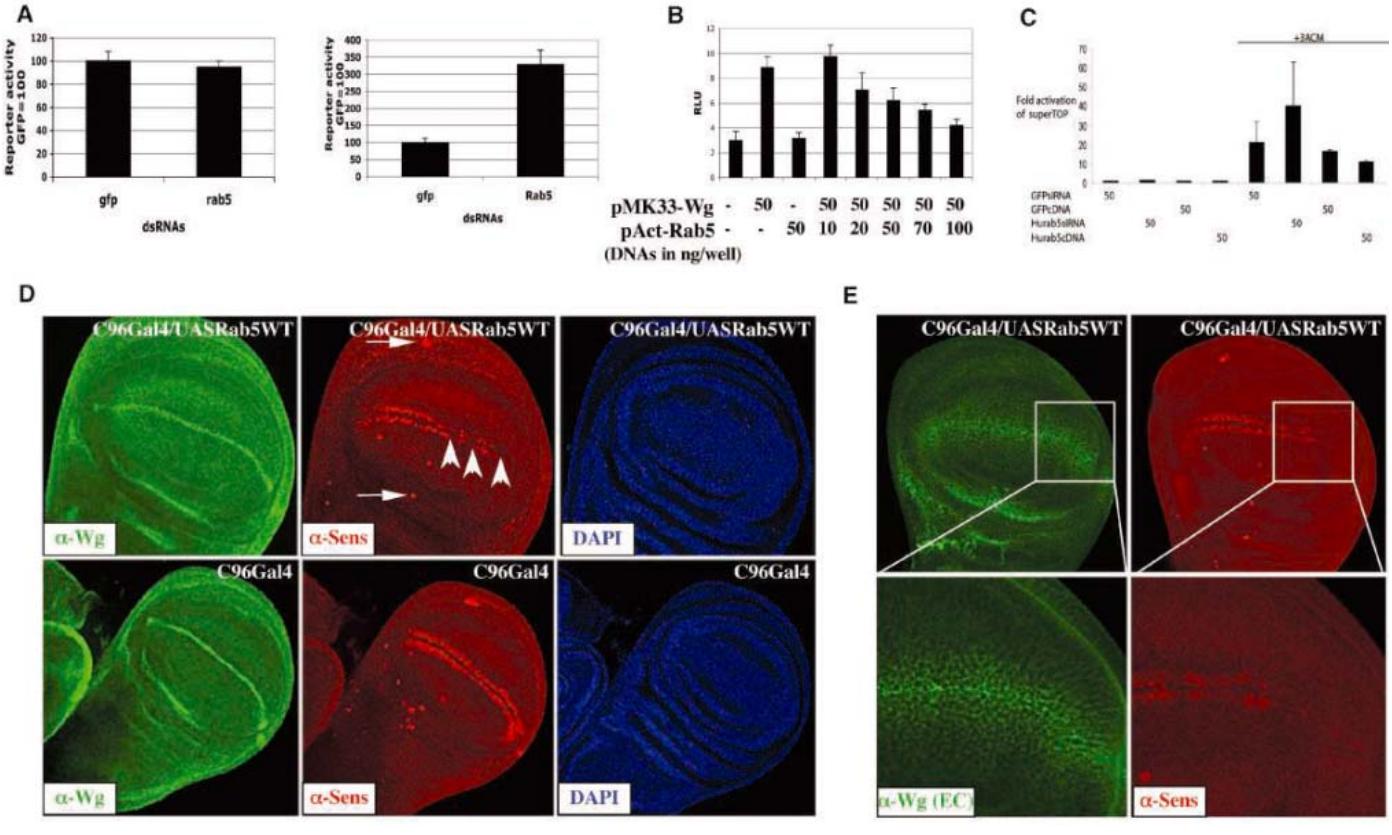
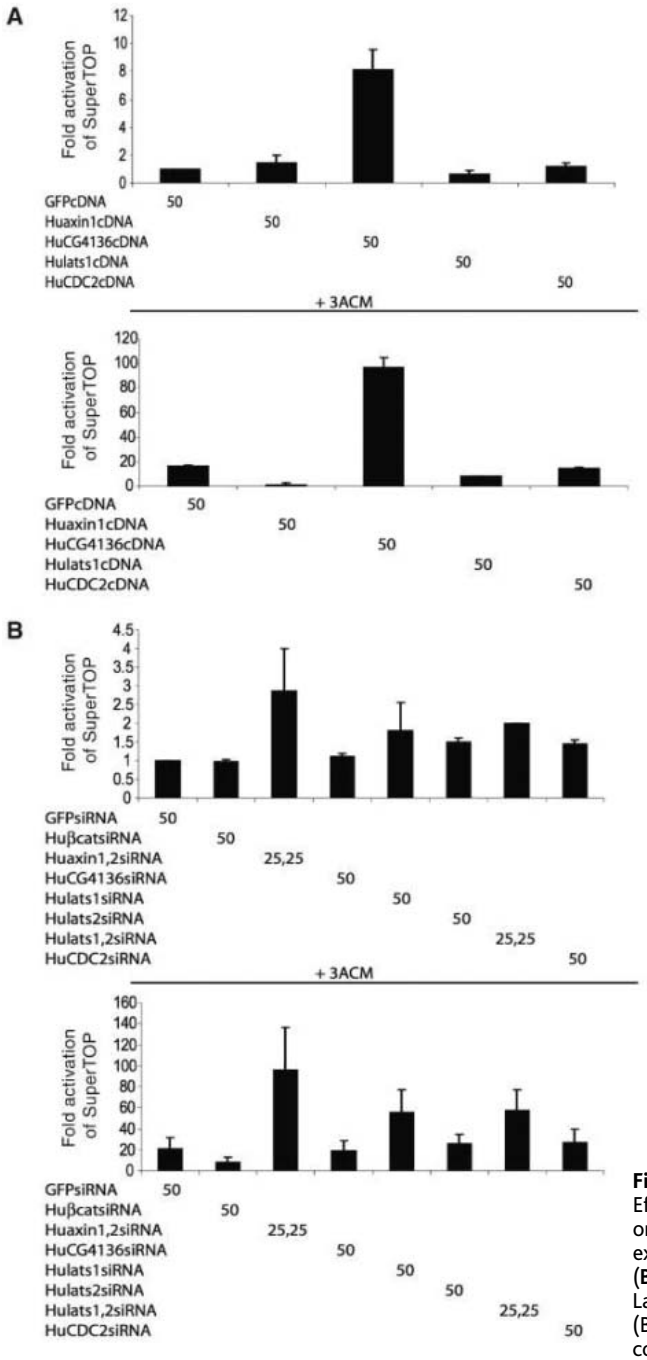
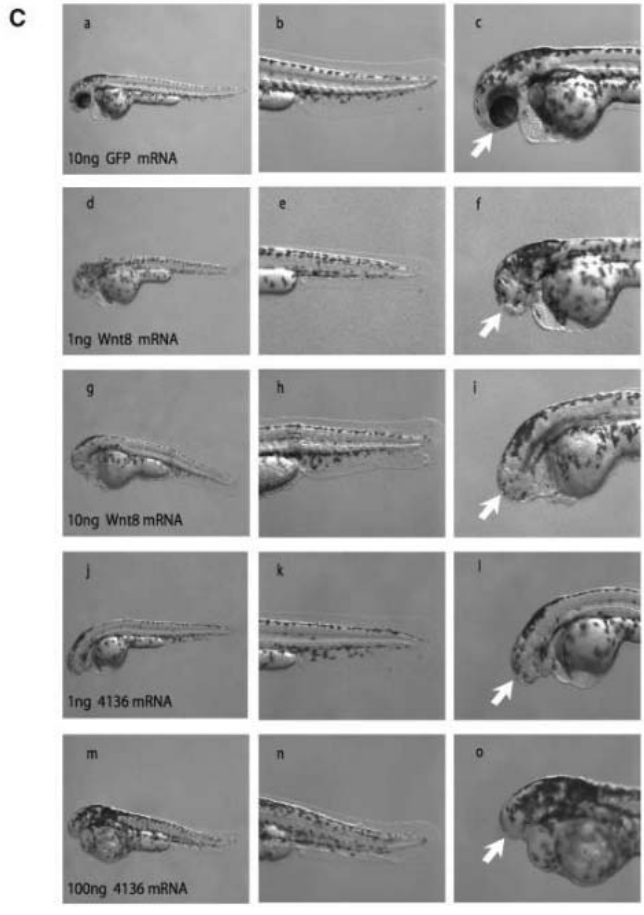


Fig. 4. Effect of Rab5 RNAi and overexpression in vitro and in vivo on the Wg signaling pathway. (**A**) Effect of Rab5 knockdown on reporter activity in control cells (left) or cells expressing Wg (right). (**B**) Rab5 overexpression in S2 cells results in a dose-dependent repression of Wg-induced reporter activity. (**C**) Effect of Rab5 overexpression and siRNA knockdown in mammalian 293T cells. (**D**) Effect of overexpression of wild-type (WT) Rab5 (upper panels) in the wing margin of the larval imaginal disc on expression of Senseless (Sens) or Wg. Control discs (lower panel). (**E**) Failure of Rab5 overexpression to change amounts of extracellular Wg protein in regions of diminished Senseless expression. DAPI (4′-6-diamidino-2-phenylindole), which forms fluorescent complexes with natural dsDNA, was used to mark the nuclei of cells in the imaginal discs.

were also stimulated with Wg (table S4 and Fig. 4A). Conversely, cotransfection of increasing amounts of Rab5 cDNA together with the Wg cDNA in *Drosophila* cells displayed a dose-dependent repression of Wg-mediated TOP-flash reporter activity (Fig. 4B). The effect on STF reporter activity in mammalian 293T cells upon Rab5 overexpression and small interfering RNA (siRNA)–mediated knockdown was similar to the effects obtained in fly cells in culture (Fig. 4C).

To assess whether Rab5 could similarly affect Wg signaling in vivo, we used the GAL4-UAS (upstream activation sequence) system to drive the expression of wild-type *rab5* in the *Drosophila* wing imaginal disc with a specific wing-margin driver, C96-GAL4 (Fig. 4, D and E). We monitored the expression of *senseless*, a proneural gene that is a target of the Wg signaling pathway at the wing margin (straddling the dorsal-ventral boundary) as a readout for pathway activity. Overexpression of Rab5 (C96GAL4-UASRab5WT) resulted in a partial to complete loss of *senseless* expression at the wing margin (Fig. 4D, arrowheads) compared with that in control discs (C96GAL4). Expression of *wg* itself was not affected (Fig. 4D). Nor was expression of *senseless* in the proneural clusters at the distal regions of the wing pouch (Fig. 4D, arrows). Because Rab5 has been implicated in receptor-mediated endocytosis and degradation of morphogenetic signals, we thought overexpression





Phenotype	GFP (10ng)	Wnt8 (1ng)	Wnt8 (10ng)	4136 (10ng)	4136 (100ng)
wild-type	60(97%)	5(12%)	2(3%)	2(3%)	5(10%)
small eyes	2(3%)	10(27%)	3(2%)	10(18%)	1(2%)
no eyes	0(0%)	27(64%)	55(92%)	45(79%)	43(88%)
Notochord defect	0(0%)	2(5%)	12(20%)	1(2%)	5(10%)

Fig. 5. Validation of candidate genes in mammalian cells and zebrafish. (**A**) (Top) Effect of overexpression of the human versions of Axin, CG4136, Lats1, and CDC2 on the activation of 16XSuperTopFlash in 293T cells. (Bottom) Same as above except with activation of the pathway with Wnt3A-conditioned media (3ACM). (**B**) (Top) Effect of knockdown of the human versions of β-cat, Axin, CG4136, Lats1, Lats2, and CDC2 on the activation of 16XSuperTopFlash in 293T cells. (Bottom) Same as above except with activation of the pathway with Wnt3A-conditioned media (3ACM). (**C**) Effect of overexpresssion of zebrafish Wnt8 ORF1 (panels d to i) or human CG4136 (panels j to o) mRNA on the development of zebrafish at 48 hours post fertilization. A couple of lateral views are shown to highlight the loss of anterior structures, such as the eye, from embryos injected with both Wnt8 and CG4136 [arrowheads in panels i and l] as compared with wild-type eyes in the GFP mRNA–injected embryos [arrow in panel c]. (Below) Table of the various phenotypes of zebrafish injected with Wnt8 ORF1 or CG4136 mRNA.

of Rab5 might influence endocytosis of the endogenous Wg protein and thus might alter signaling activity at the plasma membrane, but antibody staining against extracellular Wg revealed no difference in the levels of secreted Wg protein between regions that displayed high and low levels of *senseless* expression (Fig. 4E, insets). Thus, Rab5 appears to have a role in the control of Wg signaling activity in which it acts to inhibit Wg-dependent activation of target genes.

Our observations suggest that overexpression of Rab5 does not affect the extracellular distribution of Wg protein per se. It is possible that Rab5 could be perturbing the distribution of the receptors and coreceptors Fzd2 and Arrow (Lrp6). However, any significant change in the distribution of receptors is unlikely based on our analysis of extracellular Wg and previous studies that have demonstrated the role of Frizzled-2 receptor in regulating extracellular distribution of Wingless and shaping the Wg gradient in the wing imaginal disc (*63*). Nonetheless, we cannot rule out subtle changes at the level of receptors and/or coreceptors. Alternatively, Rab5 could be regulating trafficking of the stabilized pool of Arm/β-cat, which is present only in a Wg-induced cell and thus affecting the downstream Wingless readout as judged by antibody staining for Senseless.

Validation of mammalian orthologs in 293T cells and the zebrafish embryo. All major components of the Wnt pathway are conserved in metazoans. To determine whether the *Drosophila* genes newly identified in the RNAi screen are bona fide components of the conserved Wnt-Wg pathway, we tested their signaling activity and functions in vertebrates. We used gain and loss of function of selected vertebrate orthologs of *Drosophila* genes to assess effects on Wnt signaling in human cells and in developing zebrafish embryos.

We cloned multiple human orthologs and performed Wnt–β-cat–responsive reporter assays in human embryonic kidney (HEK) 293T cells. Transfection of plasmids encoding human Lats (also called Warts in *Drosophila*), a serine-threonine kinase, cyclin-dependent kinase 2 (CDC2), or Axin1 (as a control) inhibited the ability of Wnt-3a to activate the Wnt–β-cat–responsive reporter STF16 in 293T cells (Fig. 5A). Conversely, expression of the human ortholog of *CG4136*, a pair-like homeobox gene, activated the Wnt pathway in the presence or absence of Wnt-3A (Fig. 5A). We also generated three to four short-interfering RNAs (siRNAs) against human Lats1, Lats2, CDC2, and CG4136. Transfection of plasmids encoding pools of siRNA for β-cat inhibited Wnt-3A activation of STF16 as expected (fig. S2). Transfection of pools of siRNAs for Axin1 and 2, Lats1, Lats2, both Lats1 and 2, and CDC2 all increased basal Wnt–β-cat–responsive reporter activity and synergized with Wnt-3A activation of the reporter (Fig. 5B). Pools of siRNA for human CG4136 (HuCG4136) had no effect on activation of the Wnt–β-cat–responsive reporter (Fig. 5B), although one caveat is that 293T cells may not express HuCG4136. Because the gain of function of HuCG4136 gave a strong activation of the Wnt–β-cat–responsive reporter, it is clear that it can regulate Wnt–β-cat signaling and strongly implicates it as a new positive regulator of Wnt signaling in vertebrates. Because all of the vertebrate orthologs tested thus far affect Wnt–β-cat signaling, we are in the process of generating expression constructs and siRNAs for multiple additional human orthologs to test their roles in Wnt signaling (*64*).

To determine whether any of the vertebrate orthologs function in the Wnt pathway in vivo at the organismic level, we performed both gain- and loss-of-function assays for some of the genes that we had validated in 293T cells. For gain of function, one cell–stage zebrafish embryos were injected with RNAs encoding HuCG4136, *wnt-8* (as a positive control), and green fluorescent protein (GFP) (as a negative control). Embryos injected with GFP (10 ng) developed normally (Fig. 5C, panels a to c). However, embryos injected with *wnt-8* RNA (1 or 10 ng) developed anterior truncations and had either small eyes or no eyes in the majority of injected embryos (Fig. 5C, panels d to i) (*65*). Some of the embryos injected with 10 ng also had a defect in notochord formation (see table of Fig. 5C). Injection of RNA encoding HuCG4136 phenocopied injection with *wnt-8*, albeit at higher doses of RNA (Fig. 5C, panels j to o). This coupled with the reporter data in 293T cells indicates that HuCG4136 activates Wnt–β-cat signaling. Injection of RNA encoding Hulats1 or CDC2 had no obvious phenotype in zebrafish. However, depletion of zebrafish Lats1 by injection of antisense morpholino oligonucleotides gave a severe phenotype, and the embryos arrested before epiboly (*64*). A more detailed analysis of this phenotype will be required to determine whether it is a consequence of altered Wnt signaling. Taken together, the data from human cells and zebrafish strongly suggest that some of the hits from the *Drosophila* RNAi screen have a conserved role in Wnt–β-cat signaling in vertebrates.

Conclusions. In the future, global understanding of the complexities of and interplay between multiple signaling pathways will rely upon the systematic identification and functional characterization of unexpected regulators of signal transduction cascades. This combined with powerful genetic and biochemical analyses of molecular mechanisms might lead to breakthroughs in the fields of development and disease biology. In this Research Article, we present a whole-genome RNAi screen for the *Drosophila* Wnt-Wg signaling pathway, which in humans is implicated in hepatic, colorectal, breast, and skin cancers; bone density syndromes; Alzheimer's disease; and the retinal disease familial exudative vitreoretinopathy (*66*). Even though the primary screen was done in *Drosophila* cells, the majority of identified pathway modulators appear to share a conserved role in the regulation of the Wnt-Wg pathway in multiple *Drosophila* cell types and in mammalian cells, as judged by the functional validation of their vertebrate orthologs in 293T cells. This combined with the fact that 18% of the candidate genes identified in the screen have disease-related human orthologs [Blast E value $< 10^{-20}$, fig. S4; (*67*) and table S5] underscores the potential broad applicability and importance of such screens in future understanding and treatment of human disease. Finally, we demonstrated that selected hits from the RNAi screen function in Wnt-Wg signaling in vivo in both invertebrates (*Drosophila*) and vertebrates (zebrafish embryo). This approach has enabled us to assign new functions to previously known genes and to identify potential novel regulators of the Wnt pathway.

Although elucidating specific molecular mechanisms for selected candidate genes is beyond the scope of this study, our data strongly suggest that the RNAi-based screening in the *Drosophila* cell-based assay system is efficient in the identification of genes and will have far-reaching consequences in the expansion of our understanding of the Wnt-Wg pathway. Future studies elucidating the molecular mechanism of individual candidate genes in multiple cell types and model organisms will shed light on the complexities and nuances of this important signaling pathway. Finally, the cross-comparison of whole-genome RNAi screens for multiple signaling pathways, as well as the identification of specific versus common regulators, will help us better understand the multifactorial processes that regulate the intricate steps of animal development and disease states.

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MicroRNAs Regulate Brain Morphogenesis in Zebrafish

Antonio J. Giraldez,[1]* Ryan M. Cinalli,[1] Margaret E. Glasner,[2]† Anton J. Enright,[3] J. Michael Thomson,[4] Scott Baskerville,[2] Scott M. Hammond,[4] David P. Bartel,[2] Alexander F. Schier[1]*

MicroRNAs (miRNAs) are small RNAs that regulate gene expression posttranscriptionally. To block all miRNA formation in zebrafish, we generated maternal-zygotic *dicer* (MZ*dicer*) mutants that disrupt the Dicer ribonuclease III and double-stranded RNA–binding domains. Mutant embryos do not process precursor miRNAs into mature miRNAs, but injection of preprocessed miRNAs restores gene silencing, indicating that the disrupted domains are dispensable for later steps in silencing. MZ*dicer* mutants undergo axis formation and differentiate multiple cell types but display abnormal morphogenesis during gastrulation, brain formation, somitogenesis, and heart development. Injection of miR-430 miRNAs rescues the brain defects in MZ*dicer* mutants, revealing essential roles for miRNAs during morphogenesis.

MicroRNAs are evolutionarily conserved small non–protein-coding RNA gene products that regulate gene expression at the posttranscriptional level (*1–3*). In animals, mature miRNAs are ~22 nucleotides (nt) long and are generated from a primary transcript (termed pri-miRNA) through sequential processing by nucleases belonging to the ribonuclease III (RNaseIII) family. Initially, Drosha cleaves the pri-miRNA and excises a stem-loop precursor of ~70 nt (termed pre-miRNA), which is then cleaved by Dicer (*4–7*). One strand of the processed duplex is incorporated into a silencing complex and guides it to target sequences (*1*, *3*). This results in the cleavage of target mRNAs and/or the inhibition of their productive translation (*1–3*).

Several hundred vertebrate miRNAs and several thousand miRNA targets have been predicted or identified, but little is known about miRNA function during development (*1*, *2*, *8*, *9*). Clues to vertebrate miRNA function have come from several approaches, including expression analyses (*1–3*, *10–12*), computational prediction of miRNA targets (*8*, *13–15*), experimental support of predicted targets (*13*, *14*, *16*, *17*), cell culture studies (*16*), and gain-of-function approaches (*18*). These studies have led to the suggestions that vertebrate miRNAs might be involved in processes such as stem cell maintenance (*12*, *19*) or cell fate determination (*17*, *18*, *20*); however, no loss-of-function analysis has assigned a role for a particular miRNA or miRNA family in vivo, and it has been unclear how widespread the role of miRNAs is during vertebrate embryogenesis.

One approach to reveal the global role of vertebrate miRNAs is to abolish the generation of mature miRNAs with the use of *dicer* mutants. For example, *dicer* mutant embryonic stem cells fail to differentiate in vivo and in vitro (*20*), and *dicer* mutant mice die before axis formation (*19*), suggesting that mature miRNAs (or other Dicer products) are essential for early mammalian development. In zebrafish, maternal *dicer* activity has hampered the analysis of the single *dicer* gene. Mutants for the zygotic function of *dicer* (Z*dicer*) retain pre-miRNA processing activity up to 10 days postfertilization,

[1]Developmental Genetics Program, Skirball Institute of Biomolecular Medicine and Department of Cell Biology, New York University School of Medicine, New York, NY 10016, USA. [2]Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, MA 02142, USA. [3]Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK. [4]Department of Cell and Developmental Biology, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599, USA.

*To whom correspondence should be addressed. E-mail: schier@saturn.med.nyu.edu (A.F.S.); giraldez@saturn.med.nyu.edu (A.J.G.)

†Present address: Department of Pharmaceutical Chemistry, University of California, San Francisco, 600 16th Street, Box 2240, San Francisco, CA 94143–2240, USA.

presumably because of maternally contributed *dicer* (*21*). *Zdicer* mutants have no obvious defects other than a developmental delay at 7 to 10 days postfertilization, a stage when embryogenesis and major steps of organogenesis have been achieved (*21*). Hence, the global role of miRNAs during vertebrate embryogenesis is unknown. In light of these observations, we decided to generate zebrafish embryos that lack both maternal and zygotic *dicer* activity.

Generation of Maternal-Zygotic *dicer* Mutants

To eliminate all maternal contribution in *dicer* mutants, we took advantage of the germ line replacement technique (*22*). Wild-type zebrafish embryos depleted of their germ cells served as hosts for germ cells from homozygous *dicer* mutant donor embryos (fig. S1). The resulting fish were fertile even though they had a germ line that was exclusively constituted by mutant donor cells. As donors, we used *dicer*$^{hu715/hu715}$ mutants, an allele that codes for a truncated Dicer protein that disrupts the RNaseIII and double-stranded RNA (dsRNA)–binding domains (*21*). Intercrossing of fish that had a *dicer* mutant germ line generated embryos that were maternal-zygotic mutant for *dicer* (MZ*dicer*). In marked contrast to *Zdicer* mutants, MZ*dicer* embryos did not generate mature miRNAs and displayed severe morphogenesis defects.

Loss of pre-miRNA Processing in MZ*dicer* Mutants

Similar to other model systems, wild-type zebrafish embryos generate mature miRNAs from endogenous (*21*, *23*) or exogenously provided pri-miRNAs, resulting in the post-transcriptional repression of reporter genes (fig. S2). miRNAs induce the cleavage of reporter RNAs with perfectly complementary target sites (PT) in the 3′ untranslated region (3′UTR), whereas imperfectly complementary sites (IPT) result in the noneffective translation of reporter mRNAs (*24*) (fig. S3). Previous biochemical and genetic studies have shown that Dicer is required for the generation of mature miRNAs (*4*, *5*). To determine whether MZ*dicer* embryos lack mature miRNAs, we first hybridized total RNA from 1-day-old zebrafish embryos to a microarray of probes for 120 different zebrafish mature miRNAs (*10*). Although such arrays are susceptible to cross-hybridization artifacts, we observed a marked reduction of signals in MZ*dicer* mutants compared with wild-type embryos and zygotic *dicer* mutants (Fig. 1A). Of the 120 miRNA probes, 59, 35, and 9 gave a detectable signal in wild-type embryos, *Zdicer* mutants, and MZ*dicer* mutants, respectively. To test for the presence of mature miRNAs more specifically, we performed Northern blot analyses. We found that of eight miRNAs present in wild-type embryos, none was detected in MZ*dicer* mutants (Fig. 1B) (*25*). In most of these cases, the lack of processing resulted in an accumulation of the pre-miRNA (Fig. 1B). Northern analyses also suggested that the nine positive signals on the microarray probed with MZ*dicer* RNA are unlikely to be due to mature miRNAs. First, we found that one miRNA (miR-206) is processed in wild-type but not mutant embryos (Fig. 1B). Second, two miRNAs (miR-223 and miR-122a) were detectable neither in wild-type embryos nor MZ*dicer* mutants (Fig. 1B) (*25*). These results suggested that mature miRNAs were not generated in MZ*dicer* mutants.

As an additional assay for miRNA maturation in MZ*dicer* mutants, we examined the response of a green fluorescent protein (GFP) reporter (3xPT-miR-430b) containing target sites for members of the miR-430 family of

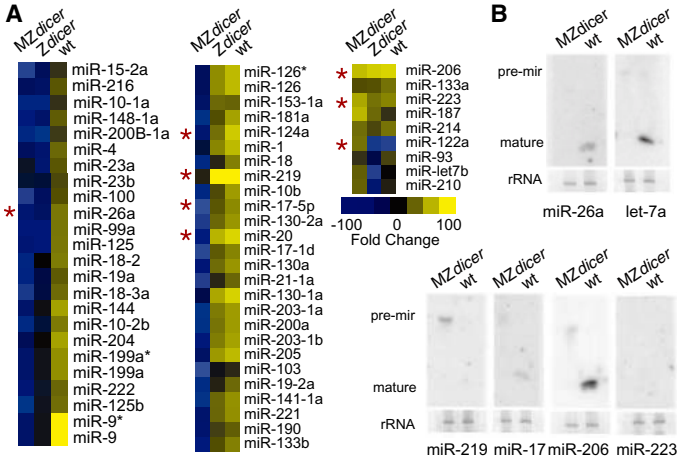

Fig. 1. MZ*dicer* mutants lack mature miRNAs. (**A**) miRNA array expression data from MZ*dicer*, *Zdicer*, and wild-type (wt) embryos at 32, 28, and 28 hpf, respectively. The range of signal was from −100-fold to 0 to +100-fold. Yellow denotes high signal and blue denotes low signal. The asterisks highlight miRNAs whose expression was also analyzed by Northern blot. (**B**) Northern blot analysis of different miRNAs in MZ*dicer* mutants (32 hpf) and wild-type embryos (28 hpf).

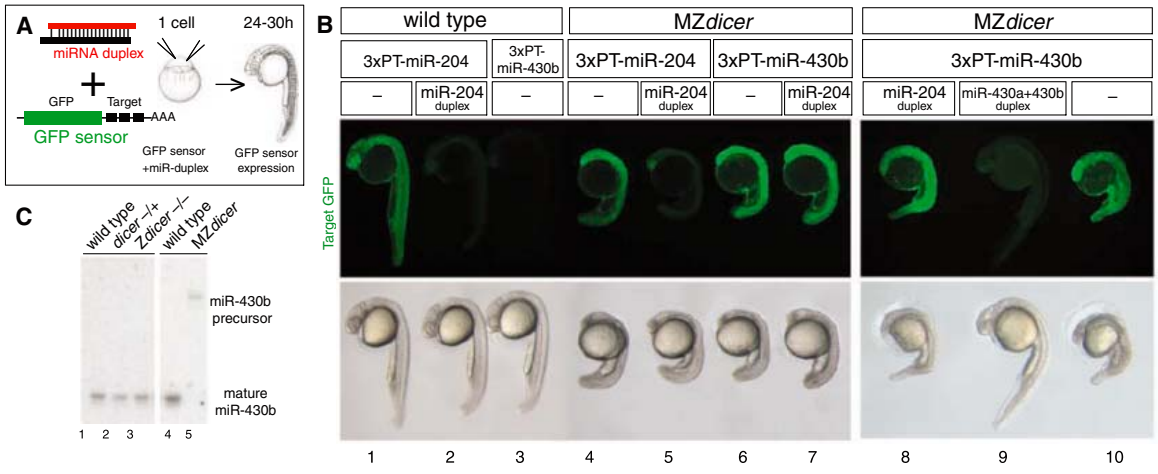

Fig. 2. Silencing activity of miRNA duplexes but not pri-miRNAs in MZ*dicer*. (**A** and **B**) Coinjection of miRNA duplexes (miR-204, miR-430a, or miR-430b) with GFP sensors that contain the coding sequence of GFP and three (3x) perfect target (PT) sites for the different miRNAs. See figs. S2 and S3 for details. (A) Schematic representation of the experimental set up. (B) Coinjection of GFP sensors with buffer (–) or miR duplexes into wild-type embryos and MZ*dicer* mutants. Fluorescent microscopy shows GFP target expression (green) at 24 to 30 hpf. Bright-field image of embryos is shown below. The specific silencing of the targets can be identified by their corresponding miRNA duplexes in wild-type and MZ*dicer* embryos. Endogenous miR-430 repressed the expression of its GFP sensor in wild-type embryos but not in MZ*dicer* mutants. (**C**) Northern blot analysis of endogenous miR-430b in wild-type and MZ*dicer* embryos, showing the accumulation of the miR-430b precursor and absence of the mature form of this miRNA in MZ*dicer* embryos.

miRNAs (Fig. 2A). These miRNAs are transcribed at high levels in the embryo and silence reporter expression in wild-type embryos (Fig. 2, B and C). In contrast, the reporter is not silenced in MZ*dicer* mutants, consistent with the lack of mature miRNAs (Fig. 2, B and C). We also injected a pri-miRNA (pri-miR-1) and followed its processing. Whereas pri-miR-1 mRNA was processed into mature miR-1 in wild-type embryos, no mature miR-1 was detected in MZ*dicer* embryos (fig. S4). Moreover, reporter genes containing perfect or imperfect targets for miR-1 were no longer silenced in MZ*dicer* mutants by pri-miR-1 injection (fig. S4). Taken together, these results indicate that MZ*dicer* mutants do not process endogenous and exogenously provided pre-miRNAs and thus are devoid of mature miRNAs.

miRNA Duplexes Are Active in MZ*dicer* Mutants

Injection of synthetic miRNA duplexes into wild-type zebrafish embryos initiates the effector step of RNA silencing and leads to the down-regulation of GFP reporters that contain complementary target sequences (*24*). Because recent studies have implicated Dicer in the assembly and function of the silencing complex (*26–30*), we analyzed the ability of exogenously provided miRNA duplexes to repress target expression in MZ*dicer* mutants. Wild-type and MZ*dicer* mutant embryos were injected with GFP sensors for three different miRNAs and with either complementary or unrelated miRNA duplexes (Fig. 2, A and B, and fig. S4). We found that miRNA duplexes specifically repressed expression of their cognate targets in both wild-type and MZ*dicer* mutant embryos (Fig. 2B and fig. S4). These results show that miRNAs can be incorporated into active silencing complexes in MZ*dicer* mutants, indicating that the RNaseIII and dsRNA-binding domains of Dicer are not required for loading into the complex and subsequent silencing activity.

Dicer Is Essential for Embryonic Morphogenesis but Not Axis Formation

The absence of mature miRNAs in MZ*dicer* mutants allowed us to determine their global requirement during early zebrafish development. The MZ*dicer* phenotype notably differs from that of Z*dicer* mutants, which are indistinguishable from wild-type embryos during these stages (*21*) (Fig. 3, A to C). Morphological analysis during the first 5 days of development revealed that axis formation and the regionalization of MZ*dicer* mutants were intact (Fig. 3C). Major subregions and cell types were present, ranging from forebrain, eye, midbrain, hindbrain, ear, pigment cells, and spinal cord to hatching gland, heart, notochord, somites, and blood (Fig. 3C and figs. S5 and S9). In contrast, morphogenetic processes during gastrulation, somitogenesis, and heart and brain development were severely affected (Fig. 3, C and E to G, and figs. S5 and S10). MZ*dicer* mutants also developed more slowly than wild-type embryos, with 3 to 4 hours of delay within the first 24 hours of development (fig. S5) (*31*).

Gastrulation. During zebrafish gastrulation, four concomitant cell rearrangements take place: (i) epiboly (spreading of the embryo over the yolk, (ii) internalization (formation of mesodermal and endodermal germ layers, (iii) convergence (movement of cells toward the dorsal side), and (iv) extension (lengthening of the embryo) (Fig. 3, E to G). MZ*dicer* mutants failed to coordinate epiboly and internalization (Fig. 3E). This resulted in mutant embryos that had undergone prechordal plate migration corresponding to 80% epiboly in wild-type embryos, yet epiboly movements were delayed to a stage equivalent to 50 to 60% epiboly (Fig. 3E). MZ*dicer* embryos also displayed a reduced extension of the axis, resulting in a shortening of the embryo and an accumulation of cells in the head region (Fig. 3, F and G). Later during development, MZ*dicer* mutants had a reduced posterior yolk extension (fig. S5).

Neural development. Neurulation was severely affected in MZ*dicer* embryos. The mutant neural plate gave rise to the neural rod, but the subsequent formation of the neurocoel and neural tube was notably impaired (Fig. 4, A, B,

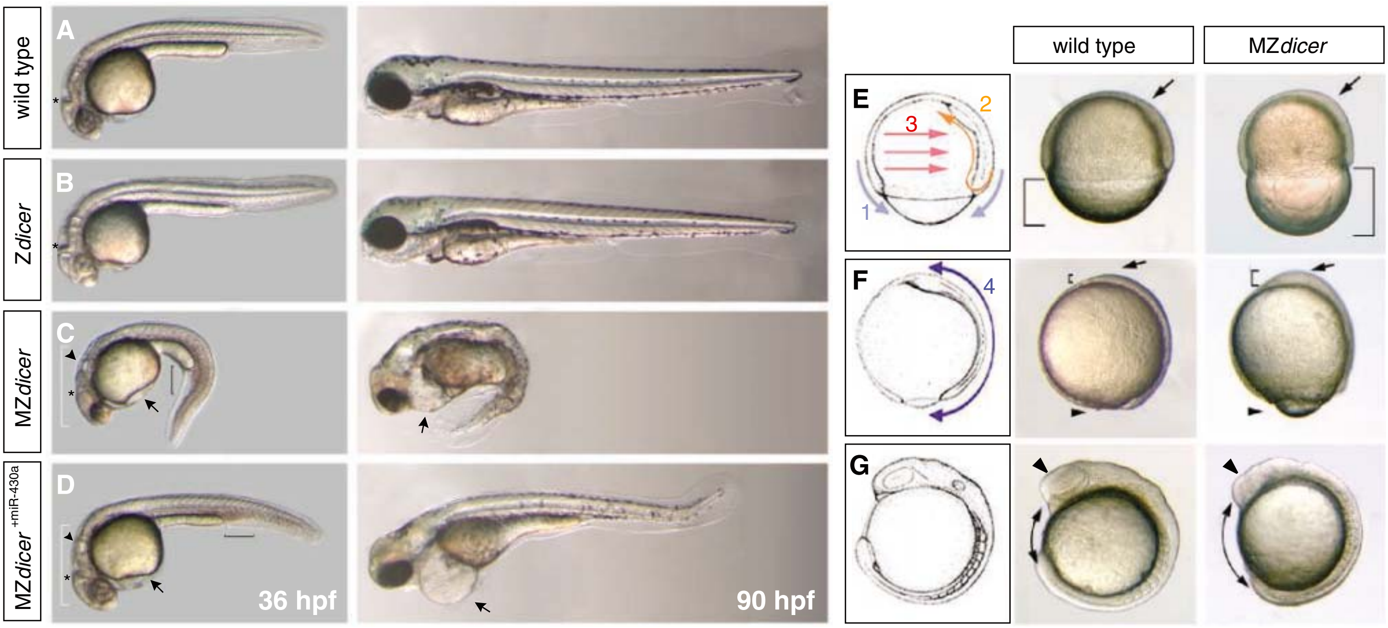

Fig. 3. Morphogenesis defects in MZ*dicer* mutants. Compared with wild-type embryos (**A**), Z*dicer* mutants (**B**) have no morphological defects at 36 and 90 hpf. (**C**) MZ*dicer* mutants display morphogenesis defects. This phenotype is fully penetrant and expressive. (**D**) MZ*dicer* injected at the one cell stage with miR-430a duplex. Brain morphogenesis (white bracket), and the midbrain-hindbrain boundary (*) are rescued, and trunk morphology and somite boundary formation are partially rescued. Phenotypes that are not rescued include the lack of blood circulation (black bracket), heart edema (arrow), and defective ear development (arrowhead). (**E** to **G**) (Left) Scheme representing the cell movements (arrows) during gastrulation in zebrafish: (1) epiboly, (2) internalization, (3) convergence, and (4) extension; (middle) wild-type embryo; (right) MZ*dicer* mutants. (**E**) Embryos at 80% epiboly stage; arrow shows the similar extent of prechordal plate extension in wild-type embryos and MZ*dicer* mutants; bracket shows a reduced extent in epiboly in MZ*dicer* mutants compared with wild-type embryos. (**F**) Tail bud stage. Note the accumulation of cells in the region of the anterior axial mesendoderm (MZ*dicer*, arrow and bracket). (**G**) Nine-somite stage. Note the abnormal development of the optic primordium (MZ*dicer*, arrowhead) and reduction in axis extension that results in a shorter embryo (MZ*dicer*, double arrow).

E, and F, and fig. S5). The formation of the brain ventricles was severely reduced. In wild-type embryos, several constrictions subdivide the brain into distinct regions. These constrictions did not form in MZ*dicer* mutants. For example, the midbrain-hindbrain boundary that is very prominent in wild-type embryos did not form in MZ*dicer* mutants (Fig. 4, B and arrowhead in F, and fig. S5). In addition, retinal development was affected (Fig. 4, A and B). Defects in the spinal cord were manifested by a rudimentary neurocoel and a reduction of the floor plate in the trunk (fig. S5).

Despite the gross morphological malformations of the nervous system, gene expression analysis suggested that anterior-posterior and dorsal-ventral patterning were not severely disrupted (fig. S6). Analysis of anterior-posterior and dorsal-ventral markers revealed normal specification of the optic stalk, forebrain, midbrain-hindbrain boundary, otic vesicles, hindbrain rhombomeres, and the dorsal and ventral neural tube.

Analysis of neuronal differentiation and axonal markers, with the use of HuC and HNK antibodies, revealed mispositioned trigeminal sensory neurons adjacent to the eye (fig. S7). In addition, we observed defasciculation of the postoptic commissure in MZ*dicer* embryos (fig. S7). In the hindbrain, multiple neurons project longitudinal axons anteriorly and posteriorly and form a ladder-like structure on each side of the midline. This scaffold was disrupted and defasciculated in MZ*dicer* mutants, but longitudinal axonal projections were established (fig. S7). In addition, touch-induced escape behavior was severely diminished in MZ*dicer* mutants (fig. S8). Taken together, these results indicate that early patterning and fate specification in the embryonic nervous system are largely unaffected by lack of miRNAs. In contrast, normal brain morphogenesis and neural differentiation and function require Dicer activity.

Nonneural development. During somitogenesis, the paraxial mesoderm becomes segmented. MZ*dicer* embryos formed normally spaced somites and expressed the muscle marker *myoD* similar to wild-type embryos (fig. S9). Later in development, the somites acquired a chevron shape in wild-type embryos but formed irregular boundaries in MZ*dicer* mutants (fig. S5). Endothelial and hematopoietic precursor cells were present as judged from the expression of the markers *fli-1* and *scl*, respectively, but endocardial *fli-1* expression was reduced and blood circulation disrupted in MZ*dicer* mutants (fig. S9). Analysis of the markers *pax2a*, *GFP-nanos-3′UTR*, *fkd1*, *cmlc2*, and *fkd2* revealed that pronephros, germ cells, endoderm, cardiomyocytes, and liver cells, respectively, were specified (fig. S6) (*25*). MZ*dicer* mutants had contractile cardiomyocytes but the two chambers characteristic of the wild-type heart did not form; instead, a tubular heart and pericardial edema developed (fig. S10).

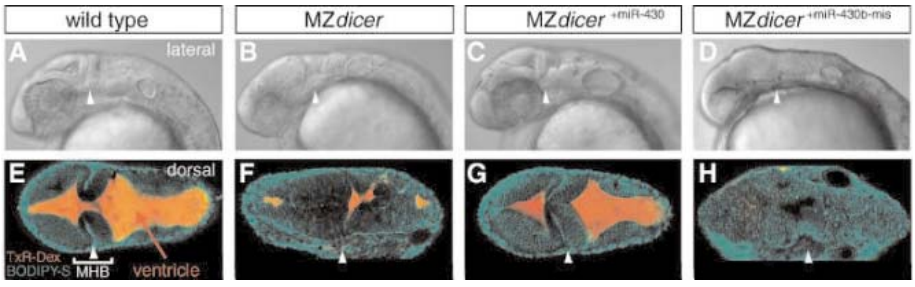

Fig. 4. miR-430 miRNAs rescue brain morphogenesis in MZ*dicer* embryos. (**A** to **D**) differential interference contrast (DIC) images of wild-type embryos (A), MZ*dicer* mutants (B), MZ*dicer* mutants injected with miR-430 duplex (MZ*dicer*$^{+miR\text{-}430}$) (C), and MZ*dicer* mutants injected with miR-430b-mis duplex (MZ*dicer*$^{+miR\text{-}430b\text{-}mis}$) (D) that contains two mismatches in the seed of miR-430b. (**E** to **H**) Confocal dorsal view of embryos with the same genotype as in (A) to (D). Cell membranes were labeled in green (BODIPY) and the brain ventricles were labeled in red by injection of Texas-Red dextran into the brain. Wild-type embryos displayed the characteristic fold of the midbrain-hindbrain boundary (MHB) (arrowhead) and have brain ventricles (red) (E). MZ*dicer* mutants do not form a midbrain-hindbrain boundary, lack normal brain ventricles, and display defects during eye development. [(C) and (G)] Injection of MZ*dicer* mutants with the miR-430 duplex rescued brain development, including the midbrain-hindbrain boundary (arrowhead) and ventricle formation. Most (84%) of the embryos were rescued (n = 104). Eye development was also partially rescued. [(D) and (H)] Injection of the miR-430b-mis duplex, which contains two mismatches in the 5′ seed, did not rescue these defects (0% rescued, n = 50).

Taken together, these results indicated that MZ*dicer* mutant embryos were patterned correctly and had multiple specified cell types but underwent abnormal morphogenesis, in particular during neural development and organogenesis.

The miR-430 miRNA Family

To identify miRNAs that might play important roles during early zebrafish development, we cloned small RNAs (~18 to 28 nt) from eight developmental stages between fertilization and 48 hours of development (*32*). These experiments identified miR-430a, miR-430b, and miR-430c as three highly expressed miRNAs, as well as several related species, miR-430d to miR-430h, which were expressed at lower levels (Fig. 5, A and B). The miR-430 family members each had the same sequence at nucleotides 2 to 8, which is known as the "seed" and has been shown to be the miRNA segment most important for target recognition (*8*, *13*, *24*, *33*). The family members also have strong homology in their 3′ region, but differ in their central and terminal nucleotides. Mapping of the miR-430 family to the zebrafish genome revealed a locus composed of multiple copies of the miR-430a,c,b triplet, with more than 90 copies of the miRNAs within 120 kb (Fig. 5C). miRNA genes are sometimes observed in clusters of about two to seven, which are frequently transcribed as a single polycistronic transcript (*34*, *35*), but the zebrafish miR-430 cluster has many more miRNAs than reported in other clusters. The miR-430 miRNAs are conserved and clustered in other fish genomes, including *Fugu rubripes* and *Tetraodon nigroviridis* (Fig. 5C). The miR-430 miRNAs belong to a superfamily that includes the vertebrate miR-17–miR-20 family, which are found in much smaller clusters in mammalian genomes (Fig. 5B). Despite the sequence similarities of the two families, members of the miR-17–miR-20 family derive from the opposite arm of their precursors, which suggest convergent rather than divergent origins of the two families. The miR-430 RNAs might share evolutionary origins with some of the miRNAs expressed specifically in mammalian embryonic stem cells (*12*), including miR-302 and miR-372, which have the same seed nucleotides and derive from the same arm of the hairpin.

The miR-430 miRNAs are initially expressed at about 50% epiboly [5 hours post-fertilization (hpf)], continue to be expressed during gastrulation and somitogenesis, and then decline at about 48 hpf (Fig. 5D) (*25*). Analysis of GFP sensors with perfect target sites for miR-430a or miR-430b suggested that the miR-430 miRNAs are ubiquitously expressed and active during early development (Fig. 2B) (*25*).

miR-430 Rescues Brain Morphogenesis in MZ*dicer* Mutants

As described above, miRNA duplexes are still active in MZ*dicer* mutants. This allowed us to determine if aspects of the MZ*dicer* mutant phenotype could be suppressed by providing specific miRNAs that are normally expressed during early zebrafish development (miR-1, miR-204, miR-96, miR-203, miR-430a, miR-430b, or miR-430c). We also reasoned that such rescue would unequivocally demonstrate that a particular phenotype is caused by the loss of a specific mature miRNA and not by the lack of small interfering RNAs (siRNAs) or

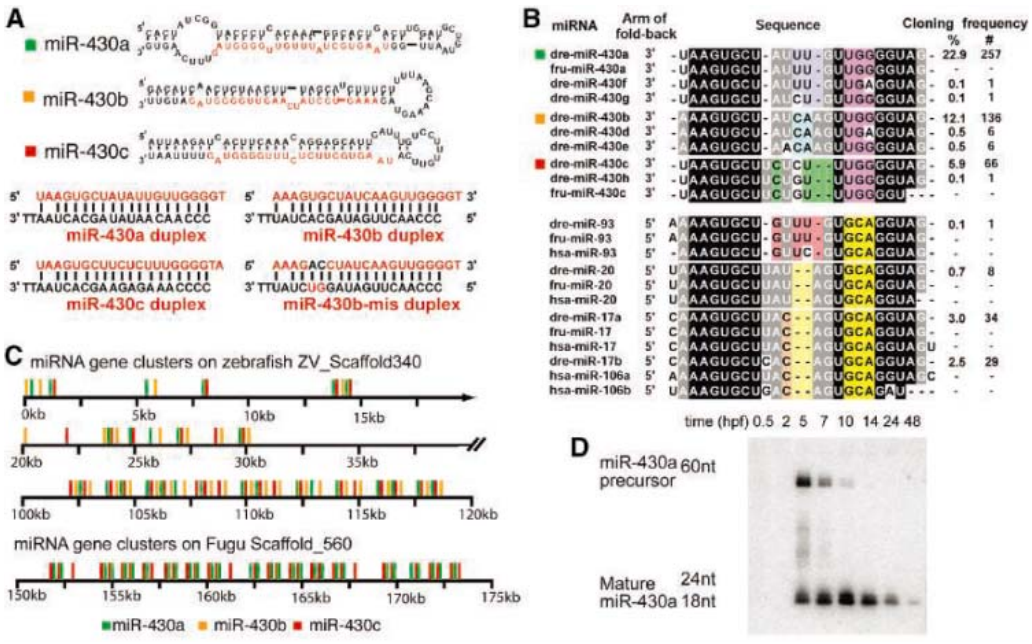

Fig. 5. Identification of a highly expressed miRNA family. (**A**) Predicted hairpins of three miR-430 miRNAs together with the corresponding duplexes used for injection; mature miRNAs shown in red. miR-430b-mis contains two mismatches (black) in the 5′ seed. (**B**) miR-430 miRNAs cloned from zebrafish (dre) and predicted in Fugu (fru) aligned with the miR-17–miR-20 family of human (hsa) miRNAs. (**C**) Color-coded representation of a miR-430 genomic cluster in the zebrafish and Fugu genomes. Each bar represents a predicted miRNA hairpin. (**D**) Northern blot analysis of the expression profile of miR-430a in wild-type embryos at different developmental stages.

the abnormal accumulation of pre-miRNAs in MZ*dicer* mutants (*5*, *7*, *20*). We found that injection of miR-430 duplexes (miR-430a, miR-430b, or miR-430c) rescued the brain morphogenesis defects in MZ*dicer* mutants (Figs. 3D and 4, C and G). This rescue was specific, as indicated by two control experiments. First, injection of unrelated miRNA duplexes did not cause any rescue (fig. S11) (*25*). Second, injection of a miRNA duplex with two point substitutions in the 5′ seed did not rescue the MZ*dicer* phenotype (miR-430b-mis; Figs. 4, D and H, and 5A; fig. S11). Rescue of MZ*dicer* mutant embryos by miR-430 (MZ*dicer*$^{+miR\text{-}430}$) resulted in normal brain ventricles and brain constrictions (Fig. 4, D and G, and fig. S11). For example, the midbrain-hindbrain boundary formed in MZ*dicer*$^{+miR\text{-}430}$ as in wild-type embryos (Fig. 4G and fig. S11). Injection of miR-430 also induced a substantial rescue of the neuronal defects observed in MZ*dicer* mutants (fig. S7). MZ*dicer*$^{+miR\text{-}430}$ also displayed partially rescued gastrulation, retinal development, somite formation, and touch response (Figs. 3D and 4C and fig. S8). In contrast, the defects in the development of the ear and heart and the lack of circulation were not rescued (Fig. 3D and fig. S10). Later during development (90 hpf), MZ*dicer*$^{+miR\text{-}430}$ embryos were developmentally delayed and displayed reduced growth similar to MZ*dicer*. These results indicate that loss of miR-430 miRNAs accounts for some but not all of the defects observed in MZ*dicer* embryos.

Our study of zebrafish that lack Dicer RNaseIII activity and mature miRNAs provides three major insights into the roles of miRNAs during embryogenesis. First, our results suggest that mature miRNAs do not have widespread essential roles in fate specification or signaling during early zebrafish development. Phenotypic comparison between MZ*dicer* mutants and embryos with aberrant signaling pathways (Nodal, Hedgehog, Wnt, Notch, CXCR4, FGF, BMP, retinoic acid, or STAT3) suggests that none of these pathways is markedly affected by the absence of miRNAs (*36*). For example, MZ*dicer* mutants do not display the phenotypes seen upon an increase or decrease in Nodal or BMP signaling. This suggests that miRNAs might have modulating or tissue-specific rather than obligatory roles in various signaling pathways. Similarly, our study reveals that MZ*dicer* mutants can differentiate multiple cell types during development. This suggests that mature miRNAs are not required to specify the major embryonic cell lineages in zebrafish. Our results do not exclude more specific roles in fate specification, such as modulating the choice between highly related cell fates. For example, lsy-6 in *Caenorhabditis elegans* controls the distinction between two closely related neurons, and mouse miR-181 seems to regulate the ratio of cell types within the lymphocyte lineage (*18*, *37*). miRNAs might also function at later stages to stabilize and maintain a particular fate. For instance, miRNAs might repress large numbers of target mRNAs to maintain tissue homeostasis by dampening fluctuations in gene expression (*38*, *39*). However, our transplantation results argue against an absolute requirement for miRNAs in every cell type. In particular, we generated fertile adults from MZ*dicer* mutant donors by germ cell transplantation (fig. S1). This indicates that primordial germ cells, the ultimate stem cells, proliferate and remain pluripotent to form the adult germ line in the absence of miRNAs. Multigeneration transplantation studies are required to determine if the lack of miRNAs has effects on germ cell maintenance (*40*, *41*). More exhaustive analysis of different cell types and signaling pathways is needed to test for more subtle or later roles of miRNAs in zebrafish, but our current study excludes a general role in signaling, embryonic fate specification, or germ line stem cell development.

Second, our results suggest important roles for miRNAs during embryonic morphogenesis and differentiation, ranging from epiboly and somitogenesis to heart, ear, and neural development. For example, loss of Dicer leads to defects in the positioning of neurons, the defasciculation of axons, and impaired touch-induced behaviors. Most notably, mutants form a neural rod but fail to generate normal brain ventricles. In addition, the morphological constrictions that subdivide the anterior-posterior axis do not form in the absence of Dicer, despite the regionalization observed by marker analysis. These results reveal essential roles of miRNAs during zebrafish morphogenesis.

Third, our study identifies a previously unknown miRNA family, the absence of which is likely to account for the brain morphogenesis defects in MZ*dicer* mutants. The miR-430 family has more genes than any miRNA family described to date, is conserved in fish, and is part of a superfamily found in other vertebrates. Injection of miR-430 duplexes suppresses the brain morphogenesis defects in MZ*dicer* mutants. This complementation approach can now be applied to determine which miRNAs (or siRNAs) account for the MZ*dicer* phenotypes that cannot be rescued by miR-430. The miR-430 family might inhibit mRNAs

that are provided maternally or expressed during early embryogenesis but are detrimental to later steps in morphogenesis. Cell shape changes, cell rearrangements, and fluid dynamics are thought to generate both extrinsic and intrinsic forces that contribute to neural tube and ventricle formation, but the underlying molecular mechanisms are poorly understood (*42*). The study of the miR-430 family and its targets therefore provides a genetic entry point to dissect the molecular basis of brain morphogenesis.

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Figs. S1 to S11
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REPORTS

The Optical Resonances in Carbon Nanotubes Arise from Excitons

Feng Wang,[1]* Gordana Dukovic,[2]* Louis E. Brus,[2] Tony F. Heinz[1]†

Optical transitions in carbon nanotubes are of central importance for nanotube characterization. They also provide insight into the nature of excited states in these one-dimensional systems. Recent work suggests that light absorption produces strongly correlated electron-hole states in the form of excitons. However, it has been difficult to rule out a simpler model in which resonances arise from the van Hove singularities associated with the one-dimensional bond structure of the nanotubes. Here, two-photon excitation spectroscopy bolsters the exciton picture. We found binding energies of ~400 millielectron volts for semiconducting single-walled nanotubes with 0.8-nanometer diameters. The results demonstrate the dominant role of many-body interactions in the excited-state properties of one-dimensional systems.

Coulomb interactions are markedly enhanced in one-dimensional (1D) systems. Single-walled carbon nanotubes (SWNTs) provide an ideal model system for studying these effects. Strong electron-electron interactions are associated with many phenomena in the charge transport of SWNTs, including Coulomb blockade (*1*, *2*), Kondo effects (*3*, *4*), and Luttinger liquid behavior (*5*, *6*). The effect of Coulomb interactions on nanotube optical properties has remained unclear, in spite of its central importance both for a fundamental understanding of these model 1D systems (*7–9*) and for applications (*7*, *10*, *11*). Theoretical studies suggest that optically produced electron-hole pairs should, under their mutual Coulomb interaction, form strongly correlated entities known as excitons (*12–18*). Although some evidence of excitons has emerged from studies of nanotube optical spectra (*7*, *19*) and excited-state dynamics (*20*), it is difficult to rule out an alternative and widely used picture that attributes the optical resonances to van Hove singularities in the 1D density of states (*21–23*). Here, we demonstrate experimentally that the optically excited states of SWNTs are excitonic in nature. We measured exciton binding energies that represent a large fraction of the semiconducting SWNT band gap. As such, excitonic interactions are not a minor perturbation as in comparable bulk semiconductors, but actually define the optical properties of SWNTs. The importance of many-body effects in nanotubes derives from their 1D character; similar excitonic behavior is also seen in organic polymers with 1D conjugated backbones (*24*).

We identified excitons in carbon nanotubes using two-photon excitation spectroscopy. Two-photon transitions obey selection rules distinct from those governing linear excitation processes and thereby provide complementary insights into the electronic structure of excited states, as has been demonstrated in studies of molecular systems (*25*) and bulk solids (*26*). In 1D materials like SWNTs, the exciton states show defined symmetry with respect to reflection through a plane perpendicular to the nanotube axis. A Rydberg series of exciton states describing the relative motion of the electron and hole, analogous to the hydrogenic states, is then formed with definite parity with respect to this reflection plane. The even states are denoted as 1*s*, 2*s*, 3*s*, and so on, and the odd wave functions are labeled as 2*p*, 3*p*, and so on (*27*). Because of the weak spin-

[1]Departments of Physics and Electrical Engineering, [2]Department of Chemistry, Columbia University, 538 West 120th Street, New York, NY 10027, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: tony.heinz@columbia.edu

orbit coupling in SWNTs, all optically active excitons are singlet states, with the allowed transitions being governed by electric-dipole selection rules. For the dominant transitions polarized along the nanotube axis, one-photon (linear) excitation requires the final and initial states to exhibit opposite symmetry. In contrast, a two-photon transition is allowed only when the final state has the same parity as the initial state. Given the symmetry of the underlying atomic-scale wave functions, one-photon excitation produces only excitons of *s*-symmetry, whereas two-photon excitation leads only to excitons of *p*-symmetry (*28*). Thus, one-photon transitions access the lowest lying 1*s* exciton; two-photon transitions access only the excited states of the exciton.

An experimental method to determine the energies of the ground and excited exciton states follows immediately from these symmetry arguments: We measured the energies needed for one-photon and two-photon transitions in semiconducting nanotubes (Fig. 1A). A comparison of these energies yields the energy difference between the ground and excited exciton states and thereby directly indicates the exciton binding strength. When the excitonic interactions were negligible, we reverted to a simple band picture in which the onset of two-photon absorption coincides with the energy of one-photon absorption (Fig. 1B). The two-photon excitation spectra reflect the qualitative difference between these two pictures in an unambiguous fashion. In contrast, conventional linear optical measurements, such as absorption and fluorescence spectroscopy, access only one-photon transitions, for which a van Hove singularity and a broadened excitonic resonance exhibit qualitatively similar features. Because the one-photon absorption and emission arise from the same electronic transition in SWNTs, there is no Stokes shift between the two, as apparent in comparison of absorption and fluorescence spectra (*8*).

In our experiment, we used isolated SWNTs in a poly(maleic acid/octyl vinyl ether) (PMAOVE) matrix. SWNTs grown by high-pressure CO synthesis were dispersed in an aqueous solution of PMAOVE by a sonication method (*29*). In order to minimize infrared absorption of water, we formed a film of SWNTs imbedded in polymer matrix by slowly drying a drop of the solution. The SWNT samples obtained by this procedure showed fluorescence emission comparable to that of the SWNTs in aqueous solution.

Two-photon excitation is a nonlinear optical effect that requires the simultaneous absorption of a pair of photons. Femtosecond laser pulses provided the high intensities of light necessary to drive this process. The light source, a commercial optical parametrical amplifier (Spectra Physics OPA-800C), pumped by an amplified mode-locked Ti:sapphire laser, produced infrared pulses of 130-fs duration at a 1-kHz repetition rate. Peak powers exceeding 10^8 W were obtained over a photon energy range from 0.6 to 1.0 eV. Because these photon energies were well below the 1-photon absorption threshold (>1.2 eV) of the relevant SWNTs, no linear excitation occurred. A laser fluence of 5 J/m^2 was typically chosen for the measurements. At this fluence, we explicitly verified the expected quadratic dependence of the excitation process on laser intensity.

To detect the two-photon excitation process in the SWNTs, we did not directly measure the depletion of the pump beam. Rather, we used the more sensitive approach of monitoring the induced light emission. The scheme can thus be described as two-photon–induced fluorescence excitation spectroscopy. Prior studies have shown that rapid excited-state relaxation processes in SWNTs (*20*) lead to fluorescence emission exclusively from the 1*s*-exciton state. Measurement of the two-photon–induced fluorescence thus yielded (Fig. 1A) both two-photon absorption spectra (from the fluorescence strength as a function of the laser excitation wavelength) and the one-photon 1*s*-exciton spectra (from the fluorescence emission wavelength). Further, because the fluorescence peaks reflect the physical structure of the emitting nanotubes, we obtained structure-specific excitation spectroscopy even when probing an ensemble sample. We detected the fluorescence emission in a backscattering geometry, using a spectrometer with 8-nm spectral resolution and a 2D array charge-coupled device (CCD) detector. Our data sampled the infrared excitation range in 10-meV steps.

The measured two-photon excitation spectra (Fig. 2) show the strength of fluorescence emission as a function of both the (two-photon) excitation energy and the (one-photon) emission energy. From the 2D contour plot, distinct fluorescence emission features emerge at emission energies of 1.21, 1.26, 1.30, and 1.36 eV (Fig. 2, circles). These emission peaks have been assigned, respectively, to SWNTs with chiral indices of (7,5), (6,5), (8,3), and (9,1) (*7*). It is apparent that none of the nanotubes were excited when the two-photon excitation energy was the same as the emission energy (Fig. 2, solid line). Only when the excitation energy was substantially greater than the emission energy did two-photon absorption occur. This behavior is a signature of the presence of excitons with significant binding energy and is incompatible with a simple band picture of the optical transitions.

The two-photon excitation spectra for nanotubes of given chiral index can be obtained as a horizontal cut in the contour plot of Fig. 2, taken at an energy corresponding to 1*s*-exciton emission of the relevant SWNT. To enhance the quality of the data, we applied a fitting procedure (*30*) to eliminate background contributions from the emission of other nanotube species. The resulting two-photon excitation spectra are shown for the (7,5), (6,5), and (8,3) SWNTs in Fig. 3. For each of the SWNT structures, the energy of the 1*s* fluorescence emission is indicated by an arrow.

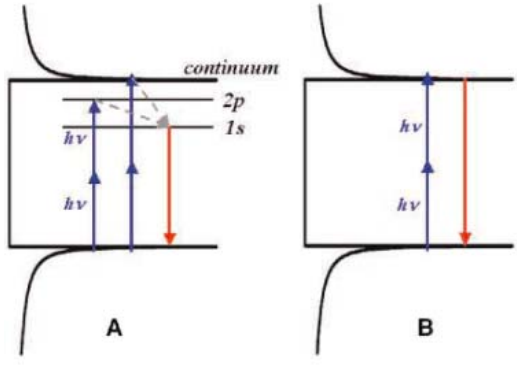

Fig. 1. Schematic representation of the density of states for a SWNT, showing the two-photon excitation (blue arrows) with photon energy $h\nu$ and subsequent fluorescence emission (red arrows) in the exciton and band pictures. (**A**) In the exciton picture, the 1*s* exciton state is forbidden under two-photon excitation. The 2*p* exciton and continuum states are excited. They relax to the 1*s* exciton state and fluoresce through a one-photon process. (**B**) In the band picture, the threshold for two-photon excitation lies at the band edge, where the relaxed fluorescence emission also takes place.

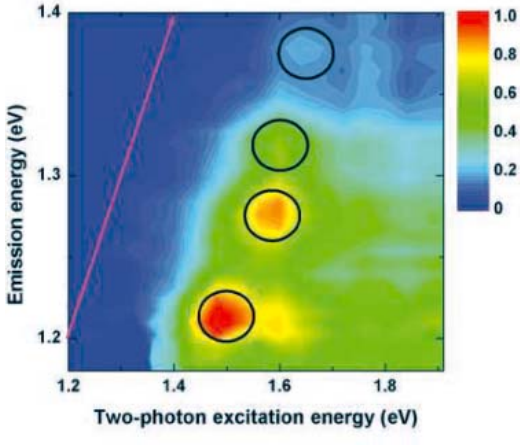

Fig. 2. Contour plot of two-photon excitation spectra of SWNTs. The measured fluorescence intensity is shown in a false-color representation as a function of the (two-photon) excitation energy and the (one-photon) fluorescence emission energy. Fluorescence peaks of different SWNT species [(7,5), (6,5), (8,3), and (9,1) with increasing emission energy] can be identified (black circles). The two-photon excitation peaks are shifted substantially above the energy of the corresponding emission feature, as is apparent by comparison with the solid line describing equal excitation and emission energies. The large shift arises from the excitonic nature of SWNT optical transitions.

The peaks in the two-photon excitation spectra can be assigned to the energy for creation of the 2*p* exciton, the lowest lying symmetry-allowed state for the nonlinear excitation process. From a comparison of this energy with that of the 1*s*-exciton emission feature, we obtained directly the relevant energy differences for the ground and excited exciton states: $E_{2p} - E_{1s}$ = 280, 310, and 300 meV, respectively, for the (7,5), (6,5), and (8,3) SWNTs.

To determine the exciton binding energy and understand the nature of the two-photon spectra more fully, we considered the two-photon excitation process in greater detail. In addition to two-photon transitions to the 2*p* state, higher lying bound excitons are also accessible (such as 3*p* and 4*p*). The strength of these transitions was relatively small, and they do not account for the main features of the spectrum. We also, however, have transitions to the continuum or unbound exciton states. Including the influence of electron-hole interactions on the continuum transitions, we found that the expected shape of this contribution to the two-photon excitation spectrum could be approximated by a step function near the band edge (*31*). The experimental two-photon excitation spectra can be fit quite satisfactorily to the sum of a Lorentzian 2*p* exciton resonance and the continuum transitions with a broadened onset.

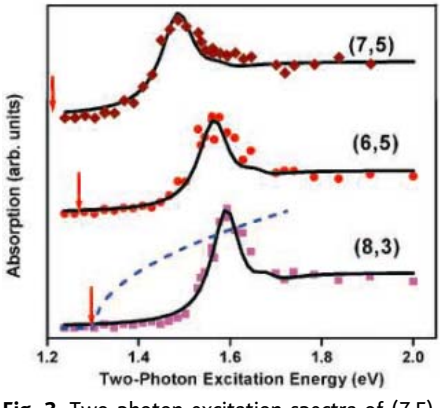

Fig. 3. Two-photon excitation spectra of (7,5), (6,5), and (8,3) SWNTs. The traces, offset for clarity, show onset energies for two-photon transitions that are appreciably higher than the corresponding fluorescence peaks (indicated by the arrows). The solid lines are the fits to the excitation spectrum obtained from our exciton model. For comparison, we show the single-particle band model prediction for an (8,3) nanotube as the dashed line in the lower trace.

A more quantitative description of the two-photon excitation spectra can be achieved with a specific model of the effective electron-hole interaction within a SWNT. In the model, we consider a truncated 1D Coulomb interaction given by the potential $V(z) = -e^2/[\varepsilon(|z| + z_0)]$ for electron-hole separation z. The value of $z_0 = 0.30d$ is fixed to approximate the Coulomb interaction between two charges distributed as rings at a separation z on a cylindrical surface of diameter d (*27*); the effective dielectric screening ε is the only adjustable parameter in the analysis. This simple model provides a good fit to the experimental data for the different nanotube species examined when we use an effective dielectric constant of 2.5 (Fig. 3, solid line). The features predicted in the model have been broadened by 80 meV (full width at half maximum). This broadening is in part experimental, reflecting the spectral width of the short laser excitation pulses (30 meV). The main contribution, however, is the width of the excitonic transition itself. This width is ascribed to lifetime broadening associated with the rapid relaxation of the excited states to the 1*s* exciton state (*20*). From this analysis, we determined the energy of 2*p* for the three SWNT species in Fig. 3 to be $E_{2p} \approx -120$ meV with respect to the onset of the continuum states at the band gap energy E_g.

Combining the previously determined $E_{2p} - E_{1s}$ energy difference with the position of the 2*p* exciton relative to the continuum, we obtained an overall binding energy for the ground-state (1*s*) exciton of $E_{ex} = (E_g - E_{1s}) \approx$ 420 meV for the investigated SWNTs. This value is comparable to recent theoretical predictions of large exciton binding energies (*13*, *14*). The exciton binding energy thus constitutes a substantial fraction of the gap energy $E_g \approx 1.3$ eV for our 0.8-nm SWNTs. To put this result in context, the exciton binding energies in bulk semiconductors typically lie in the range of several meV and represent a slight correction to the band gap. Furthermore, because thermal energies at room temperature exceed typical bulk exciton binding energies, excitonic effects in bulk materials can be largely neglected under ambient conditions. This situation clearly does not prevail for SWNTs.

We can understand the strong increase in excitonic effects in the SWNTs as the consequence of two factors. The first arises from a general property of reduced dimensionality: In three dimensions, the probability of having an electron and hole separated by a displacement of r includes a phase space factor of r^2, favoring larger separations over smaller ones. In one dimension, no such factor exists. Short separations are thus of greater relative importance, and the role of the Coulomb interactions is enhanced. The second factor relates to the decreased dielectric screening for a quasi-1D SWNT system. This effect arises because the electric field lines generated by the separated electron-hole pair travel largely outside of the nanotube, where dielectric screening is decreased. Because these effects are general features arsing from the 1D character, they should be widely present in 1D systems. Indeed, similar excitonic effects have been extensively studied in a large family of 1D structures of conjugated polymers (*24*).

To help visualize the strongly bound excitons in SWNTs, we estimated the exciton's spatial extent, i.e., the typical separation between the electron and the hole in the correlated exciton state. Assuming an exciton kinetic energy comparable to its binding energy E_{ex}, which applies precisely for 3D excitons, we obtain the relation $E_{ex} \sim \hbar^2/2mR^2$, where $\hbar$ is Planck's constant h divided by 2π, m is the reduced electron-hole mass, and R is the exciton radius. For $m = 0.05\ m_0$ (*21*), we deduced from our experimental binding energy a ground-state exciton radius of $R = 1.2$ nm. This value is similar to that obtained by calculation within the truncated Coulomb model specified above. Figure 4 provides a representation of the calculated density distribution of the exciton envelope wave function. The result is a highly localized entity, with a spatial extent along the nanotube axis only slightly exceeding the nanotube radius of 0.8 nm.

The importance of excitonic effects is clear for the interpretation and assignment of the observed optical spectra, as discussed in the literature on the relation of the E_{11} and E_{22} transition energies in SWNTs (*7*, *15*, *17*). The excitonic character of the optically excited state also has immediate implications for optoelectronic devices and phenomena. For example, photoconductivity in SWNTs should have a strong dependence on the applied electric field, because charge transport requires spatial separation of the electron-hole pair. The excitonic character of optically excited SWNTs also raises the possibility of modifying the SWNT transitions through external perturbations, thus facilitating new electro-optical modulators and sensors. More broadly, the strong electron-hole interaction demonstrated in our study highlights the central role of many-body effects in 1D materials.

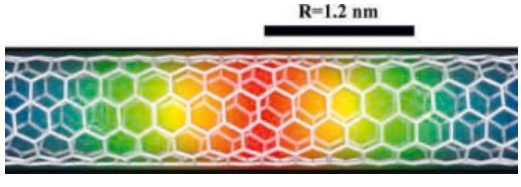

Fig. 4. Density of the 1*s*-exciton envelope wave function for a (6,5) SWNT. The wave function has been calculated using the experimentally determined exciton binding energy and the truncated Coulomb electron-hole interaction. The density represents the probability of finding the electron and hole composing the exciton at the indicated relative separation. The half width of the exciton along the nanotube is $R = 1.2$ nm, compared to the 0.8-nm diameter of the nanotube.

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30. To eliminate the influence of background in determining the two-photon excitation spectrum of SWNTs of a given chiral index, we plotted the experimental emission spectra, corresponding to vertical cuts in the two-dimension contour plot of Fig. 2, for a series of two-photon excitation energies. We then fit each emission spectrum to a sum of Lorentzian features corresponding to the relevant nanotube species in our ensemble sample. The two-photon excitation spectrum for a given nanotube chiral index was then obtained by tracking the peak height of corresponding fluorescence contribution as a function of the two-photon excitation energy.
31. For the continuum states in a 1D direct-gap material, the two-photon absorption cross section σ_{TPA} scales as $\sigma_{TPA} \propto (E - E_g)^{1/2}$ within the free carrier picture, where E denotes the photon energy and E_g the bandgap energy. This form is modified by strong electron-hole interactions. Within the Wentzel-Kramers-Brillouin approximation, one can show generally that this correction leads to an enhancement near the band edge that produces a step function for the two-photon cross-section, $\sigma_{TPA} \propto \theta(E - E_g)$, where θ is the usual Heavyside function. This correction is analogous to the well-known result for one-photon excitonic transitions in bulk semiconductors (*32*).
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Zircon Thermometer Reveals Minimum Melting Conditions on Earliest Earth

E. B. Watson[1]* and T. M. Harrison[2,3]

Ancient zircons from Western Australia's Jack Hills preserve a record of conditions that prevailed on Earth not long after its formation. Widely considered to have been a uniquely violent period geodynamically, the Hadean Eon [4.5 to 4.0 billion years ago (Ga)] has recently been interpreted by some as far more benign—possibly even characterized by oceans like those of the present day. Knowledge of the crystallization temperatures of the Hadean zircons is key to this debate. A thermometer based on titanium content revealed that these zircons cluster strongly at ~700°C, which is indistinguishable from temperatures of granitoid zircon growth today and strongly suggests a regulated mechanism producing zircon-bearing rocks during the Hadean. The temperatures substantiate the existence of wet, minimum-melting conditions within 200 million years of solar system formation. They further suggest that Earth had settled into a pattern of crust formation, erosion, and sediment recycling as early as 4.35 Ga.

The first 500 million years of Earth evolution, a period known as the Hadean Eon, was the most geodynamically vigorous in our planet's history. During this time, it is variously speculated that the Earth may have experienced collision with a Mars-sized object (*1*), formed a global magma ocean (*2*), grown the first continents (*3*), and seen the emergence of life (*4*). It is also entirely possible, and consistent with the geochemical record, that none of these events took place. The fundamental problem is that we have no rock record from this interval to learn about these processes because the oldest firmly dated rock is 4.04 Ga (*5*). How, then, are we to gain further insights into the formative stages of Earth evolution?

[1]Department of Earth and Environmental Sciences, Rensselaer Polytechnic Institute, Troy, NY 12180, USA. [2]Research School of Earth Sciences, Australian National University, Canberra, ACT 2601, Australia. [3]Department of Earth and Space Sciences and Institute of Geophysics and Planetary Physics, University of California, Los Angeles, CA 90095, USA.

*To whom correspondence should be addressed. E-mail: watsoe@rpi.edu

Although no Hadean rocks are yet documented, we are not entirely without a geochemical record of the period between 4.5 and 4.0 Ga. The existence of zircons >4.1 Ga preserved in Early Archean metasediments at Mt. Narryer and Jack Hills, Western Australia, has been known for more than 20 years (*6*, *7*), and recent measurements have begun to glean information from them regarding the nature of the Hadean Earth. For example, Hf isotopic studies suggest the existence of reworked continental crust before 4.1 Ga (*8*). Oxygen isotope results have been interpreted as indicating that protoliths of ~4.3-Ga magmas formed in the presence of water at the Earth's surface (*9*, *10*). Xenon isotopic studies of these ancient zircons have permitted an estimate of the initial terrestrial plutonium/uranium ratio, a parameter key to understanding the origin and evolution of the atmosphere (*11*).

These and other results have challenged the traditional view that continental formation and development of a hydrosphere were frustrated by meteorite bombardment and basaltic igneous activity until ~4 Ga. Instead, they suggest a surface environment and petrogenetic processes much more similar to those of the present day. Here, we exploit a newly developed thermometer, based on Ti incorporation into crystallizing zircon, to assess the nature of Hadean magmatism. From these analyses, we conclude that Jack Hills zircons were dominantly sourced from crustal melts that formed at temperatures ranging from those characteristic of wet, minimum melting to vapor absent melting under anatectic conditions.

Titanium content is uniquely suitable as a potential indicator of zircon crystallization temperature. As a tetravalent ion under all relevant geologic conditions, Ti enters the zircon lattice in homovalent replacement of Zr^{4+} or Si^{4+}. Consequently, Ti uptake does not depend on the availability of other charge-compensating ions. For the TiO_2-saturated case (i.e., rutile present in the system), the thermodynamic basis of the thermometer is the simple reaction

$$TiO_{2(\text{rutile})} = TiO_{2(\text{zircon})} \qquad (1)$$

for which the equilibrium constant is

$$k_1 = \frac{a^{\text{zircon}}_{TiO_2}}{a^{\text{rutile}}_{TiO_2}}$$

where a_{TiO_2} is the activity of TiO_2 in rutile or zircon as indicated by the superscript. Because rutile is nearly pure TiO_2, $a^{\text{rutile}}_{TiO_2} \sim 1$, so

$k \cong a_{TiO_2}^{zircon}$. Therefore

$$a_{TiO_2}^{zircon} = \gamma_{TiO_2}^{zircon} \cdot X_{TiO_2}^{zircon} = \exp \frac{-\Delta G_1^o}{RT} \quad (2)$$

where γ is the activity coefficient and X the mole fraction of TiO_2 in zircon, ΔG_1^o is the standard-state free-energy change for reaction 1, R is the gas constant, and T is absolute temperature. Assuming $\gamma_{TiO_2}^{zircon}$ is constant, the logarithm of the Ti concentration in zircon is expected to be linear in T^{-1}. Confirmation and quantification of this relation would constitute a crystallization thermometer for zircon in the presence of rutile.

The Ti thermometer was calibrated experimentally at 1025° to 1450°C (1 to 2 GPa) and by analysis of natural zircons known to have crystallized at ~580° to 1070°C on the basis of independent geothermometers. We used a piston-cylinder apparatus to grow zircons in the presence of rutile, both from aqueous solution and by crystallization from silicate melt (*12*). The Ti concentrations in these synthetic zircons were determined by electron microprobe analysis and range between ~100 parts per million (ppm) (1025°C) and ~1300 ppm (1450°C). The natural zircons were separated from five well-characterized rocks: the Bishop Tuff (*13*); a rutile-bearing migmatite from the Adirondack Mountains (*14*); a rutile-bearing aluminous schist from the Tauern Window in the Eastern Alps [Stillup Tal (*15*)]; a rutile-bearing metasomatic vein in a harzburgite nodule from Labait volcano in Tanzania (*16*); and a rutile-bearing migmatite from a mafic subduction complex exposed at Santa Catalina Island, California (*17*). Cathodoluminescence (CL) imaging confirmed a simple crystallization history for the Bishop Tuff (BT) zircons; those from the Adirondack migmatite (ADK), the Alpine schist (ST), and the California migmatite (SC) have inherited cores with CL-dark overgrowth rims of varying width believed to have formed at or near peak metamorphic conditions. Zircons in the Labait harzburgite (LB) are large euhedra (up to 500 μm) with CL zoning ranging from concentric to patchy. We determined Ti concentrations in these zircons (or rims) using an ion microprobe (see below) for the ADK, SC, ST, and BT cases and an electron microprobe for the more Ti-rich LB case. Analyses of ADK, SC, ST, and LB zircons are used directly in the calibration because rutile is present in the host rocks. In the BT case, however, the measured Ti contents of the zircons were adjusted upward slightly, in accordance with the subunity TiO_2 activity in the system, estimated to be 0.6 from the Ti contents of abundant quartz phenocrysts cocrystallized with the zircons (*18*) (note that the BT does contain ilmenite). The overall thermometer calibration (Fig. 1) conforms well to prediction (Eq. 2), spans almost 900°C in temperature, and shows little sensitivity to pressure. Application of this thermometer to zircons of unknown crystallization temperature requires simple measurement of Ti content.

Strictly speaking, the Ti-in-zircon thermometer applies to systems containing rutile. Accordingly, the temperatures measured must be regarded as minimum values unless cocrystallization with rutile can be established. This

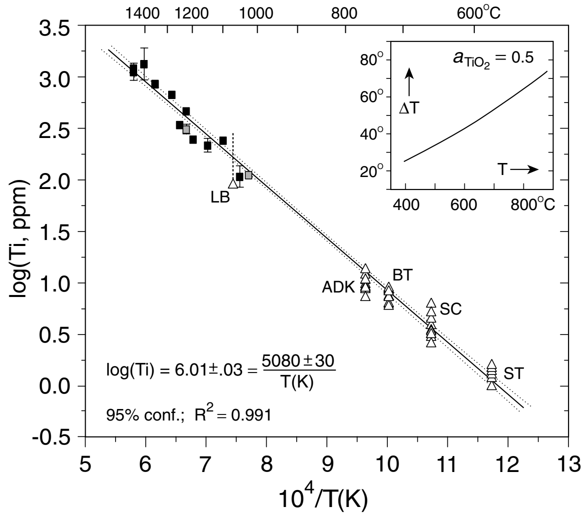

Fig. 1. Temperature dependence of Ti incorporation into synthetic zircons (black and gray squares are at 1 and 2 GPa, respectively) and zircons separated from natural rocks (triangles). The natural samples are Bishop Tuff (BT) (*13*), Adirondack migmatite (ADK) (*14*), Stillup Tal aluminous schist (ST) (*15*), Labait harzburgite (LB) (*16*), and Santa Catalina migmatite (SC) (*17*). All systems contain rutile except BT, which contains ilmenite (see text). For the natural zircons analyzed by ion microprobe (ADK, BT, SC, and ST), individual analyses rather than mean values are plotted. The variation in Ti content of zircons from a specific rock is well outside the analytical uncertainty (*23*) and thus may reflect real variation in growth temperature (the ion microprobe lacks the spatial resolution to systematically traverse a zircon rim). The data are plotted at the temperatures estimated from other thermometers (or at the midpoint when a range is indicated). In the LB case, the triangle represents the mean of ~90 spot analyses in the interiors of two zircons; the Ti content is higher in patches near the margins of these zircons, as indicated by the dotted line extending upward from the plotted symbol. Pyroxene thermometry places the temperature of the host at 1070°C (*37*), but a brief, late heating event is recorded by the pyroxene rim compositions. The uncertainty in the best-fit line is indicated in the figure; this translates into an uncertainty of ±10°C (2σ) at relevant application temperatures. The inset illustrates the extent to which the Ti content of zircons would underestimate temperature (by Δ*T*) if the activity of TiO_2 in the system were 0.5.

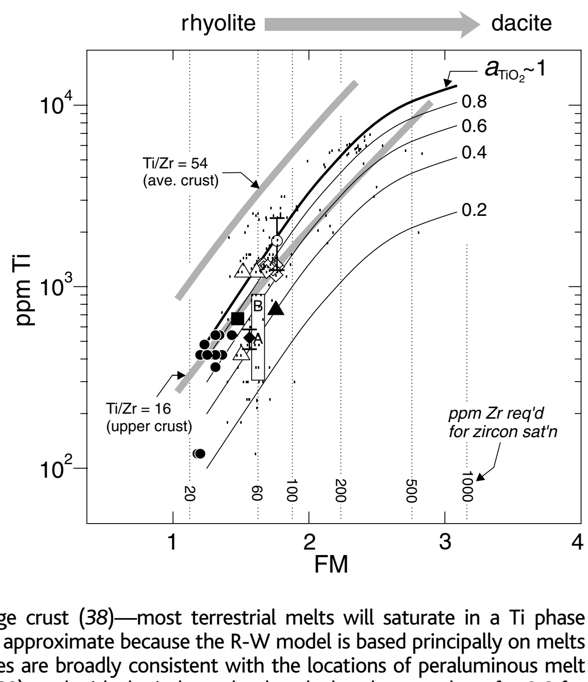

Fig. 2. Analyses of silicic glass inclusions and volcanic glasses in relation to estimated TiO_2 activity in magmatic melts based on the rutile saturation model of Ryerson and Watson (*20*) for 0.1-GPa pressure. The heavy black curve labeled "$a_{TiO_2} \sim 1$" delineates Ti concentrations sufficient for rutile saturation according to the Ryerson-Watson (R-W) model (fig. S1); curves for $a_{TiO_2} < 1$ were calculated assuming Henrian behavior in the melt. The vertical dotted lines indicate the approximate amount of Zr required for zircon crystallization at a given value of FM and implied *T* (*21*). The diagram reveals that most natural melts capable of crystallizing zircon also have TiO_2 activities exceeding ~0.5. Because the $a_{TiO_2} \sim 1$ curve corresponds to a Ti/Zr ratio at rutile and zircon cosaturation of ~20—compared with a value of ~50 for the average crust (*38*)—most terrestrial melts will saturate in a Ti phase before zircon. The activity curves are approximate because the R-W model is based principally on melts having FM > 3; however, these curves are broadly consistent with the locations of peraluminous melt inclusions containing ilmenite (●) (*39*), and with the independently calculated a_{TiO_2} value of ~0.6 for the Bishop Tuff ("B") and ~0.4 for the Bandelier rhyolite ("A") (*18*). Other symbols: (■) melt inclusion (MI) mean, Taylor Creek rhyolite (*40*); (▲) MI mean, Alid volcanic center, Eritrea (*41*); (♦) MI mean, Paleozoic volcanic ash (*42*); (◇) mean of MI in quartz, Ordovician bentonites, New York (*43*); (△) MI in Yellowstone rhyolites (*44*); (○) MI mean, Taupo, New Zealand (*45*); vertical white bar indicates the Ti range for MI in phenocrysts from Bishop Tuff (*46*) and Bandelier rhyolite (*47*). Other volcanic glasses and MI are plotted as black specks because Ti was analyzed as part of a major-element electron microprobe routine and the values have significant uncertainty [data sources (*48*–*50*); lowest SiO_2 contents are ~65 weight %].

consideration is important for the Hadean zircons because they are removed from their original surroundings and their coexistence with rutile is generally uncertain. Fortunately, this does not appreciably weaken the constraint on zircon growth temperature provided by Ti content, for the following reasons. In metamorphic systems, a_{TiO_2} ranges from 0.6 in metabasites to ~1 in metapelites (*19*). In igneous systems, a_{TiO_2} is also broadly constrained by existing knowledge of the rutile saturation surface (*20*). For a wide variety of magmatic melts, the same factors that lead to high activities of ZrO_2 (resulting in zircon saturation) (*21*) also lead to high activities of TiO_2. Saturation in zircon and rutile depend weakly on pressure and strongly on temperature and melt composition, where the latter is expressed as a ratio of cation fractions: $FM = (Na^+ + K^+ + 2R^{2+})/(Al^{3+} \cdot Si^{4+})$, where R = Ca for the case of zircon (*21*) and R = Ca + Fe + Mg for rutile (*20*). If the Hadean zircons are magmatic, as is clearly the case for those with oscillatory zoning (see below), the range of possible host-melt compositions and temperatures is quite restricted—by virtue of the presence of zircon—for plausible levels of dissolved Zr. Broadly speaking, the limitations on melt composition and temperature imposed by the presence of zircon itself restrict a_{TiO_2} to values generally >0.5 (Fig. 2). Only peralkaline melts are exempt from this general reasoning, because of the high solubilities of zircon (*22*) and the lack of systematic data on rutile solubility.

In summary, for most igneous and metamorphic rocks in existence today, a_{TiO_2} is 0.5 or higher. The host materials of the Hadean zircons cannot be assumed a priori to resemble those typical of more recent times, but their characteristics were governed by the same thermodynamic considerations. Given that a_{TiO_2} generally is ≥0.5, the crystallization temperatures of most of these zircons will not be underestimated by more than 50° to 60°C (Fig. 1, inset).

Using an ion microprobe (*23*), we measured Ti concentrations in 54 Jack Hills concordant zircons ranging in U-Pb age from 4.0 to 4.35 Ga. At least one analysis spot on each zircon was chosen to coincide with the locations where the ages were determined. Calculated temperatures from 69 spots range from 801° to 644°C (696° ± 33°C) (Fig. 3). In most cases, duplicate Ti determinations on single zircons yielded similar temperatures; however, one zircon fragment with CL zoning suggesting a simple magmatic history shows a systematic diminution in crystallization temperature from 778°C near the core to 751°C near the rim (Fig. 4). This pattern is consistent with progressive zircon growth during cooling of the host magma.

The most notable feature of these results is the low and restricted range of temperatures which, taken at face value, implies water-saturated melting conditions. Before we explore this possibility, we first examine two alternative scenarios. First, could the zircon temperature distribution result from cooling of melts derived from the expected high flux of impacting bolides? We rule out this possibility for the following reasons: (i) The melting temperature in the Qz-Ab-Or-H_2O system, even in the presence of the 270-bar steam atmosphere resulting from complete evaporation of the ocean (*24*), exceeds 800°C (*25*); (ii) the dispersion of the temperature distribution is low (Fig. 3), implying a dominant, regulated melting mechanism (this is especially true if the eight outliers in the distribution at T > 750°C are attributed to an alternative mechanism); and (iii) zircon saturation temperatures calculated for magmas produced by wholesale melting of average crust (*26*) exceed the average Hadean zircon temperatures we observe. Second, could the Hadean zircon temperature distribution reflect residual liquids that might have fractionated from higher temperature, mafic magmas? We believe this scenario is ruled out by the expectation that late-stage crystallization in a mafic complex would yield appreciably higher average temperatures for zircon formation. For example, calculated zircon saturation temperatures for late differentiates of the Skaergaard intrusion yield an average temperature of 870°C (*27*, *28*). Our thermometer gave temperatures of 787° to 806°C for three Skaergaard zircons analyzed in this study (these are probably biased downward slightly by undersaturation of the system in rutile). Moreover, although fractionation of mafic magma can indeed yield felsic melts and intermediate-temperature zircons, this mechanism does not seem to meet the requirement of a regulated mechanism suggested by our data.

Thus, we believe that our crystallization temperatures for the Hadean zircons provide definitive evidence about the state of the early Earth, especially when considered in the context of the inclusion assemblage documented by other workers. Minerals contained within Hadean zircons include quartz, K-feldspar, chloritized biotite, chlorite, amphibole, muscovite, albite, Ca-Al silicate (plagioclase), rutile, apatite, FeOOH, Ni-rich pyrite, thorite (ThO_2), and monazite (*29*, *30*). Collectively, these inclusions are characteristic of granitic assemblages or their hydrated and/or oxidized equivalents (*29*). Until now, the possibility has remained that the inclusion assemblage may represent siliceous, feldspathic material of an-

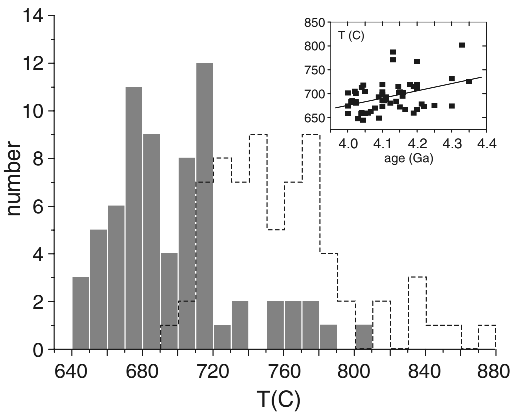

Fig. 3. Histogram of crystallization temperatures for Hadean zircons derived from measured Ti contents and thermometer calibration in Fig. 1. The distribution represented by the gray bars assumes a_{TiO_2} = 1; the dashed line shows the shift to somewhat higher temperatures for a_{TiO_2} = 0.5. The inset shows the distribution of zircon crystallization temperatures over time.

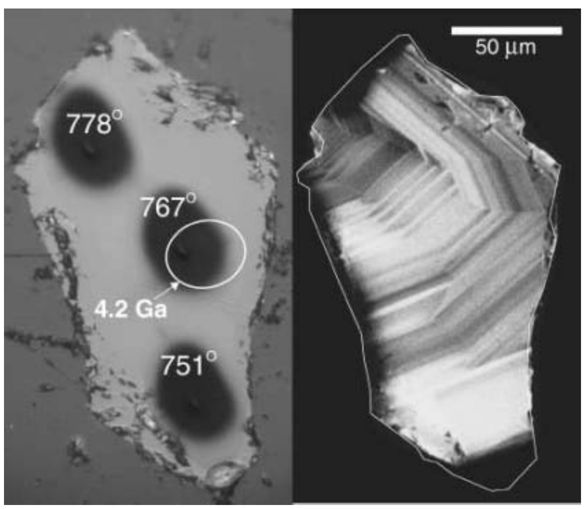

Fig. 4. (**Left**) Reflected-light image of Hadean zircon fragment ANU104-14.14, Au-coated for ion microprobe analysis. The white ellipse shows the location of the ion-microprobe analysis pit that yielded a concordant U-Pb age of 4.2 Ga. The dark spots reveal locations of ion-microprobe Ti analyses, from which temperatures were calculated using the information in Fig. 1. (**Right**) Cathodoluminescence image of the same zircon fragment, showing igneous growth zoning from core (top left) to rim (bottom); the thin white line is the fragment outline from the left photo. Note the correspondence of falling temperatures on the left with progressive growth on the right.

hydrous character that was altered by later exposure of the zircons to crustal metamorphic fluids. The temperatures measured by our Ti thermometer provide strong evidence against this possibility: Even with allowances for subunity TiO_2 activity (Fig. 2), they are simply too low for the zircons to have crystallized from dry siliceous melts (*31*). The restricted range of temperatures suggests, furthermore, that a highly reproducible set of circumstances removed melt fertility from rocks under prograde conditions consistent with crustal anatexis throughout the Hadean. Temperatures for zircons >4.2 Ga are sparse, but the present database hints at a slight down-temperature "focusing" of typical magmatic conditions between 4.35 and 4.0 Ga (Fig. 3, inset).

The simplest scenario is melting in an ensemble of crustal environments not unlike that of today under conditions at or close to water saturation. Taken collectively, our zircon crystallization temperatures mimic expectations for "modern-day" igneous zircons, with most pointing to a crustal anatectic origin.

The present results substantiate the existence of wet, minimum melting conditions at 4.35 to 4.0 Ga inferred from mineral inclusion studies and are consistent with the early Hadean hydrosphere hypothesis (*9*, *10*). They strongly suggest, moreover, that within ~100 million years of formation, Earth had settled into a pattern of crust formation, erosion, and sediment recycling similar to that produced during the known era of plate tectonics. The rapid establishment of this cycle implies, further, that the pace of geologic activity in general (driven by rapid mantle convection) was much faster in the Hadean than in more recent times.

References and Notes

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An Octane-Fueled Solid Oxide Fuel Cell

Zhongliang Zhan and Scott A. Barnett*

There are substantial barriers to the introduction of hydrogen fuel cells for transportation, including the high cost of fuel-cell systems, the current lack of a hydrogen infrastructure, and the relatively low fuel efficiency when using hydrogen produced from hydrocarbons. Here, we describe a solid oxide fuel cell that combines a catalyst layer with a conventional anode, allowing internal reforming of *iso*-octane without coking and yielding stable power densities of 0.3 to 0.6 watts per square centimeter. This approach is potentially the basis of a simple low-cost system that can provide substantially higher fuel efficiency by using excess fuel-cell heat for the endothermic reforming reaction.

Improving fuel efficiency is one of the key reasons, along with reduced pollution, for the adoption of fuel cells for applications such as transportation. Improving efficiency not only reduces fuel consumption but also reduces the associated CO_2 emission. Although fuel cells can achieve efficiencies of 50 to 60%, overall "well-to-wheels" efficiencies are cur-

rently only ~29% (*1*) because of the relatively low efficiency of hydrocarbon conversion (i.e., reforming) to H_2, where excess heat must be added. The efficiency may improve to as high as 42% as the technology improves but still may not provide sufficient motivation to replace lower-cost options such as gasoline-electric hybrids with efficiencies of ~32%. Furthermore, H_2 production from hydrocarbons releases as much CO_2 as does direct use in an internal combustion engine, and H_2 is more expensive than gasoline (*2*, *3*). (Hydrogen production from renewable energy sources is not considered here; although this will be a truly pollution-free solution, it will not provide a substantial fraction of global energy needs in the foreseeable future.)

However, there is a well-known method to improve well-to-wheels fuel-cell efficiency to ~50% (*4*) by using excess fuel-cell heat for the endothermic reforming reaction. Solid oxide fuel cells (SOFCs) are well suited for this because their operating temperatures are high enough to provide the high-temperature heat needed for reforming (*5*). There has been considerable recent interest in SOFCs for efficient auxiliary electrical power generation for heavy- and light-duty vehicles, as well as aircraft (*6*, *7*). With reductions in SOFC operating temperature, there may also be interest in battery/SOFC hybrid technology for vehicle propulsion, in which the SOFC would serve as a battery charger with relatively infrequent cycling (frequent on-off cycling would likely be problematic for high-temperature SOFCs). A transportation SOFC would likely use internal reforming within the stack, both because the system is simplified (a separate reformer system is not needed) and because heat transfer is optimal (*8*). Unfortunately, internal reforming of typical transportation fuels, that is, gasoline and diesel, has not been successful because they cause coking on Ni-based SOFC anodes (*9*).

Here, we describe a SOFC design that combines a catalyst layer with a conventional anode that allows internal reforming of *iso*-octane without coking and yields stable power densities of 0.3 to 0.6 W/cm². *Iso*-octane, a high-purity compound similar to gasoline, was used in these experiments to achieve a demonstration of the feasibility of SOFCs with transportation fuels. This avoids a number of experimental complications with commercial fuels, which typically contain a number of different compounds and additives with a substantial range of compositions, as well as relatively large amounts of sulfur contaminants that can seriously affect fuel-cell performance. The experiments were done with conventional SOFCs at ~800°C or with low-temperature (<600°C) SOFCs that may be more suitable for transportation applications.

Many of the experiments used SOFCs similar to those being developed worldwide (*10*). These SOFCs consisted of a thin yttria-stabilized zirconia (YSZ) electrolyte layer, a thick Ni-YSZ anode support, and a composite cathode consisting of $La_{0.8}Sr_{0.2}MnO_3$ (LSM) and 8 mol% Y_2O_3-stabilized ZrO_2 (YSZ) or $La_{0.6}Sr_{0.4}Co_{0.2}Fe_{0.8}O_3$ (LSCF) and $Ce_{0.9}Gd_{0.1}O_{1.95}$ (GDC) (*4*). Without any modification, these SOFCs were unstable when used with *iso*-octane/CO_2/H_2O fuel streams. Examples of the decrease in cell voltage for constant current operation at 770°C with three different fuel compositions—6% *iso*-octane/94% CO_2, 3% *iso*-octane/72% CO_2/25% steam, or 5% *iso*-octane/76% CO_2/19% air—are shown in Fig. 1. The degradation was caused by severe coke buildup on the Ni-YSZ anode, which was visible to the eye in many cases. A scanning electron microscope energy-dispersive x-ray spectrum (fig. S2A) (*4*) taken from the SOFC anode after degradation showed a clear carbon peak along with the expected Ni, Zr, and Y peaks.

Two modifications were required to achieve stable operation with *iso*-octane fuel mixtures. First, a porous catalyst layer—an ~0.5-mm-thick piece of stabilized zirconia with a thin layer of Ru-CeO_2 on both sides (total Ru loading 2 mg/cm² in all cases)—was placed against the anode side of the SOFC. The effect of the catalyst layer can be seen by comparing curves 1 and 4 in Fig. 1, which were both obtained with 6% *iso*-octane balanced by CO_2. The cell was much more stable with the catalyst than without, showing a nearly constant voltage, although subsequent observation indicated very slight carbon deposition on the catalyst layer. Although the initial cell voltages in curves 1 and 4 are similar, the current density was lower for curve 4 because of the catalyst layer; the lower current was not the reason for the improved stability, however, because SOFC stability against coking generally degrades as current decreases (*11*). Second, the addition of a small amount of air to the fuel yielded fully stable performance (curve 5 in Fig. 1) without measurable carbon deposits detected on the catalyst layer or the fuel cell (as shown in fig. S2B) (*4*).

A differentially pumped mass spectrometer was used to observe the products of the

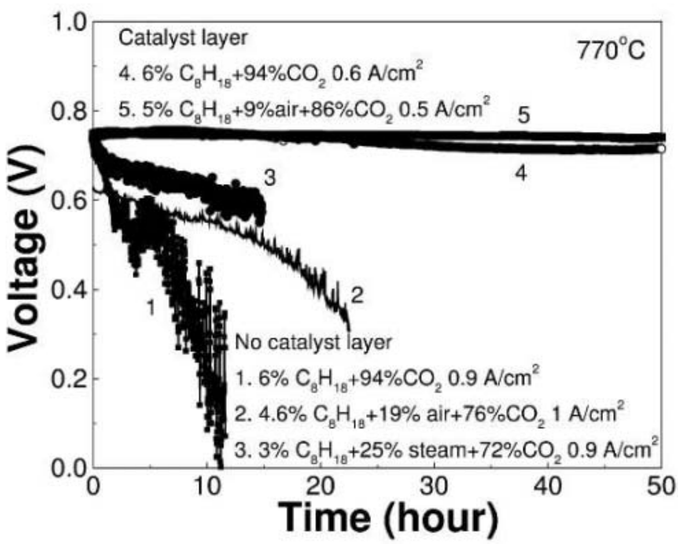

Fig. 1. Life tests of anode-supported SOFCs (Ni-YSZ|YSZ|LSM-YSZ, LSM) operated on various *iso*-octane/CO_2/steam/air mixtures with or without a catalyst layer at 770°C.

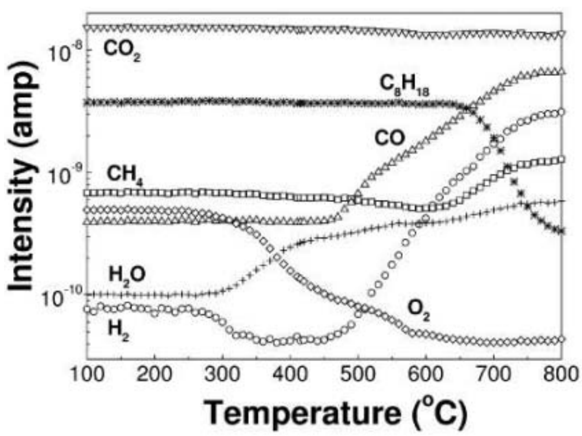

Fig. 2. Mass spectrometer peak intensities versus temperature for 5% *iso*-octane/9% air/86% CO_2 fuel mixtures after flowing over a Ru-CeO_2 catalyst layer at 100 SCCM.

Department of Materials Science and Engineering, Northwestern University, 2220 Campus Drive, Evanston, IL 60208, USA.

*To whom correspondence should be addressed. E-mail: s-barnett@northwestern.edu

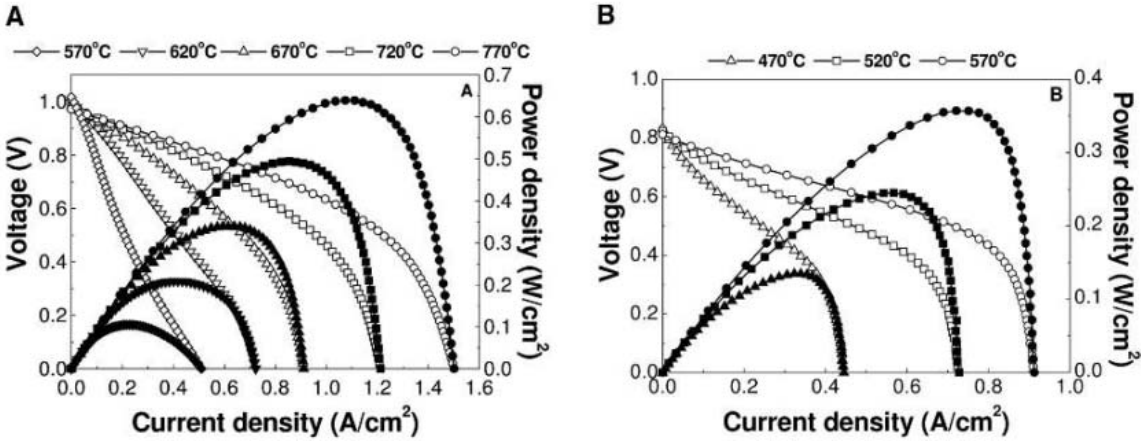

Fig. 3. Voltage and power density versus current density for the cell, tested in 5% *iso*-octane/9% air/86% CO_2 at 100 SCCM in the anode and ambient air in the cathode at different cell temperatures. (**A**) NiO-YSZ|YSZ|LSCF-GDC, LSCF, with a catalyst layer, Ru-CeO_2|PSZ|Ru-CeO_2. (**B**) NiO-SDC|SDC|LSCF-GDC, LSCF, with a catalyst layer, Ru-CeO_2|PSZ-CeO_2|Ru-CeO_2.

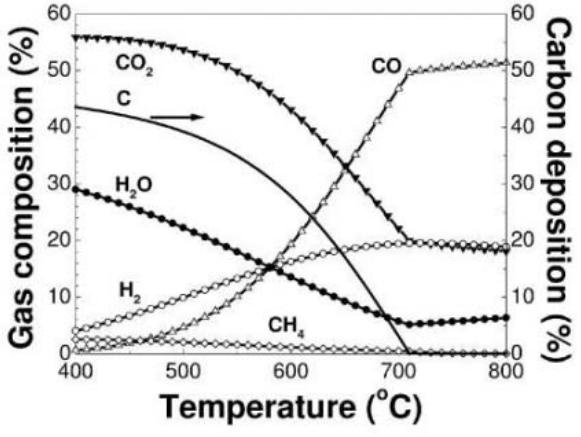

Fig. 4. Calculated equilibrium product distribution as a function of temperature for a 5% *iso*-octane/9% air/86% CO_2 inlet fuel mixture.

fuel reactions on the Ru-CeO_2 catalyst. A description of the measurement and typical mass spectra (fig. S3) are given in (*4*). For a 5% *iso*-octane/9% air/86% CO_2 mixture flowing at 100 standard cubic centimeters per minute (SCCM) over the catalyst, there was no apparent reaction below ~280°C, that is, the H_2, CO, and CH_4 peaks were at background levels whereas CO_2, *iso*-octane, and O_2 did not decrease (Fig. 2). The O_2 peak decreased with increasing temperature from ~280°C to 550°C, which indicates that *iso*-octane oxidation was occurring. No change in the *iso*-octane peak was detected in this range, because the O_2 amount was sufficient to react at most 10% of the *iso*-octane. The primary reaction products that can be used by the SOFC, CO, and H_2 did not begin to increase measurably until the temperature reached ~550°C. This change, along with the corresponding decrease in the *iso*-octane peak that became obvious above ~650°C, indicated that dry reforming was occurring. Above 700°C, a small amount of methane was observed.

Typical stable cell performance at different temperatures in *iso*-C_8H_{18}/CO_2/air with the catalyst layer (Fig. 3A) exhibited open-circuit voltages from 0.97 to 1.02 V that tended to increase with decreasing temperature, which is consistent with the thermodynamically predicted values of 0.99 to 1.03 V. The maximum power densities ranged from 0.1 W/cm² at 570°C to 0.6 W/cm² at 770°C. The cells were stable over a wide range of operating conditions, not just the high currents shown in Fig. 1. For example, cells kept at zero current for 50 hours with 5% *iso*-octane/9% air/86% CO_2 maintained a stable open-circuit voltage without coking on the anode or the catalyst layer (fig. S4) (*4*).

The results in Fig. 3A illustrate a typical trend in YSZ-electrolyte SOFCs: Power densities drop significantly below ~650°C (*12*). Lower temperature SOFCs are desired for transportation applications, so we also tested reduced-temperature SOFCs with thin Sm-doped ceria (SDC) electrolytes, thick Ni-SDC supports, and composite cathodes containing LSCF and GDC. Figure 3B shows an example of SOFC/catalyst performance with *iso*-octane/CO_2/air fuel. The catalyst layer and air addition were found to be important for maintaining stable, coke-free operation over typical 50- to 100-hour tests (figs. S5 and S6) (*4*). The SDC cells yielded maximum power densities of ~0.35 W/cm² at 570°C, a value comparable to those reported for other SDC-electrolyte cells operated on H_2 (*13*). The open-circuit voltage was relatively low for these cells because of the electronic conductivity in SDC electrolytes (*13*). The maximum power values were limited in part by the increase in slope seen in the voltage-current curves at high current (Fig. 3B). Such behavior is often attributed to limited gas transport through one or both electrodes (*14*) and is not surprising given the present thick anodes and low H_2 partial pressures below 600°C (Fig. 2). Thus, increasing the fuel H_2 content by improving the low-temperature catalyst performance is one possible means to improve upon the cell performance in Fig. 3B.

The above results showed that the catalyst layer was crucial for allowing stable cell operation without coking. Based on the thermodynamic analysis shown in Fig. 4, the present fuel compositions were stable against coking at temperatures >720°C but were within the coking regime for the lower temperature cells. Coking can still occur on Ni catalysts even under thermodynamically non-coking conditions (*9*). Recent studies have shown that Ni is especially well suited for growing carbon fibers (*15*). Coking from carbon-containing gases on metal catalysts like Fe or Co has also been observed (*16*). It is nonetheless desirable to use Ni or similar metals in SOFC anodes because they are very good electrochemical catalysts (*17*). Recent studies have used Cu as an anode conductor because it is inert against coking and reforming; unfortunately, it is not a good electrochemical catalyst, such that power densities were typically relatively low (*18*).

The present cells had conventional Ni-based anodes to achieve good power densities and a catalyst layer to prevent coking. The lack of coking at the Ni-based anode can be explained by reforming at the Ru-Ceria catalyst layer, which eliminated most of the hydrocarbon species before the fuel reached the anode. A key element of this strategy was the choice of a catalyst metal, Ru, that promotes hydrocarbon reforming but does not itself cause coking (*19*). For some of the conditions used here (<720°C), coking was expected (Fig. 4), yet no carbon was observed on the

catalyst layer in any case, even for the low-temperature experiments (fig. S6B). The coking on Ru was presumably kinetically limited.

There are three minor drawbacks to the catalyst layer. First, it is expected to reduce the rate at which fuel can diffuse to the anode, thereby decreasing cell power density. Indeed, test results at 770°C typically showed lower power densities of ~0.6 W/cm^2 with the catalyst layer compared with initial values (prior to degradation) of 0.8 to ~1 W/cm^2 without the layer. Second, because the catalyst layer was electrically insulating in the present experiments, electrical current collection could be an issue. However, a slight modification of a typical interconnect design, in which the catalyst layer is present only in the interconnect gas-flow channels as shown schematically in fig. S7 (*4*), could be used to provide good current collection. Third, Ru is expensive, although less so than precious metal catalysts such as Pt and Rh. As discussed in the supplemental materials (*4*), this cost should not be prohibitive for reasonable Ru loadings.

The other factor required for stable cell operation was having at least 10% air in the fuel mixture. This represents a 2% oxygen addition to the fuel, and it is not clear what role this plays in preventing coking. One possibility is that the oxygen helps remove carbon on the catalyst. Although the addition of oxygen amounts to burning some of the fuel, the amount of air is too small to substantially reduce the efficiency or to substantially dilute the fuel with nitrogen.

The present results compare favorably with other recently reported methods for using heavy hydrocarbon fuels in SOFCs. SOFCs have recently been reported to operate successfully on N_2-diluted gasoline (*18*) and other heavy hydrocarbon fuels (*20*). However, the power densities were substantially lower (0.1 W/cm^2) than in the present results. Prototype SOFC systems have recently been developed for vehicle auxiliary electrical power using external partial-oxidation reforming of gasoline to produce a hydrogen-rich fuel (*6*). However, partial-oxidation reforming has substantially lower efficiency than is possible with H_2O-CO_2 reforming (*21*). In cases where reforming is done within the stack but away from the cells (*22*) or in an external reformer (*23*), heat transfer is not as good and additional hardware is required. One disadvantage of direct internal reforming is that the endothermic reaction may be too rapid and may cause substantial SOFC or catalyst cooling near the fuel inlet (*24*). The present catalyst layer may have an advantage in this regard, because the catalyst material can be varied to suitably adjust the rate of the reforming reaction. The addition of oxygen to the fuel also helps mitigate this cooling because of the exothermic partial-oxidation reaction.

The present small-scale demonstrations show the feasibility of SOFCs fueled by hydrocarbons such as *iso*-octane, with substantial advantages including high efficiency, a relatively simple system design, and reduced operating temperature. However, more work needs to be done to prove that this approach is practical with real fuels such as gasoline, diesel, and aircraft fuels; recently developed techniques for reducing sulfur contamination to low levels (*25*) may be especially useful.

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From Dimming to Brightening: Decadal Changes in Solar Radiation at Earth's Surface

Martin Wild,[1]* Hans Gilgen,[1] Andreas Roesch,[1] Atsumu Ohmura,[1] Charles N. Long,[2] Ellsworth G. Dutton,[3] Bruce Forgan,[4] Ain Kallis,[5] Viivi Russak,[6] Anatoly Tsvetkov[7]

Variations in solar radiation incident at Earth's surface profoundly affect the human and terrestrial environment. A decline in solar radiation at land surfaces has become apparent in many observational records up to 1990, a phenomenon known as global dimming. Newly available surface observations from 1990 to the present, primarily from the Northern Hemisphere, show that the dimming did not persist into the 1990s. Instead, a widespread brightening has been observed since the late 1980s. This reversal is reconcilable with changes in cloudiness and atmospheric transmission and may substantially affect surface climate, the hydrological cycle, glaciers, and ecosystems.

Solar radiation at Earth's surface (also known as global radiation or insolation) is the primary energy source for life on our planet. Widespread measurements of this quantity began in the late 1950s. Trends in worldwide distributed observational records of solar radiation have been proposed in various studies (*1–5*). These studies report a general decrease of sunlight over land surfaces on the order of 6 to 9 W m^{-2} from the beginning of the measurements in about 1960 until 1990, corresponding to a decline of 4% to 6% over 30 years. Such a decrease may profoundly influence surface temperature, evaporation, the hydrological cycle, and ecosystems, as noted in (*6–10*).

Thus far, no study has addressed the evolution of solar radiation from 1990 onward, because extensive observational data after 1990 were not easily accessible. The main source for data prior to 1990 in (*1–5*) was the Global Energy Balance Archive (GEBA) (*11*), which

Table 1. Changes in surface solar radiation over Europe. Three hundred sites were merged into 32 ISCCP (*21*) equal-area grid cells over Europe. Results were obtained by fitting linear models with station effects (*2*) to annual means of surface solar radiation within each cell for two specified periods (significant trends at the 5% level are in parentheses). The period 1950 to 1990, considered in earlier studies (*1*–*5*), predominantly shows decreases in surface solar radiation, whereas increases dominate in the period 1985 to 2000. Data source: GEBA/WRDC (*11*).

	1950–1990	1985–2000
Number of cells	32	32
Increase	8 (3)	26 (8)
Decrease	24 (13)	6 (0)

we have updated for the 1990s in the present work, with support from the World Radiation Data Centre (WRDC) in Saint Petersburg, Russia. We also used surface radiation measurements from the Baseline Surface Radiation Network (BSRN) of the World Climate Research Program (WCRP), available from 1992 onward (*12*). This global network measures surface radiative fluxes at the highest possible accuracy with well-calibrated state-of-the-art instrumentation at selected sites in the major climate zones. The data in both GEBA and BSRN underwent rigorous quality checks, as described in (*11*, *12*), to assure high accuracy as well as homogeneity in the data, a prerequisite for regression analyses. Here, we evaluate the newly available surface observations to investigate changes in solar radiation in more recent years.

The most comprehensive data for the 1990s are available for the European area. Seven thousand yearly values measured at 300 stations from GEBA/WRDC were analyzed in 32 grid cells on an equal-area grid of ~2.5° resolution. The results in Table 1 were obtained by estimating linear models in each cell [including station effects, as described in (*2*)]. Out of 24 cells with a systematic decrease over the period 1950 to 1990 considered in the earlier studies (*1*–*5*), only 6 show a decrease over the period 1985 to 2000, none of them statistically significant. As can be inferred from Table 1, dimming of solar radiation fades after 1985 over Europe, and a reversal to brightening is found (*13*). On average, trends change their sign from negative to positive in 1985, as obtained from the minima of second-order linear models in 14 cells. Illustrations for the reversal from dimming to brightening at various sites in Central and Eastern Europe are found in figs. S1 to S3.

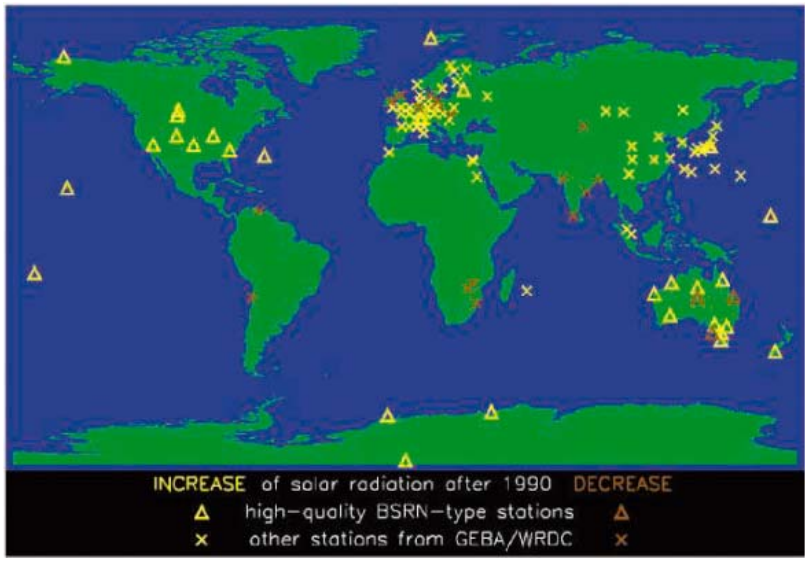

Fig. 1. Global distribution of surface observation sites used in this study. Sites measuring an increase in surface solar radiation after 1990 are marked in yellow; sites measuring a decrease are shown in brown. High-quality observation sites fulfilling the BSRN standards (*12*) are shown as triangles, other sites from the updated GEBA as crosses. Information from 300 sites over Europe and 45 sites over Japan are displayed as aggregated regional means. The majority of the sites show an increase in surface solar radiation after 1990.

The transition from decreasing to increasing solar radiation is in line with a similar shift in transparency of the cloud-free atmosphere determined from pyrheliometer measurements, which show a general tendency of decreasing atmospheric transmission up to the early 1980s and a gradual recovery thereafter (fig. S4). This may be related to a decrease of aerosol burden due to more effective clean-air regulations and the decline in the economy with the political transition in Eastern European countries in the late 1980s, as manifested, for example, in a lower local planetary albedo due to reduced aerosol loadings and related effects on clouds in these countries (*14*). Associated changes in atmospheric transmission, solar radiation, and cloudiness are documented in the long-term records from Tartu-Toravere in Estonia (figs. S3 and S4) (*15*).

In addition to these European-based observations, we found a similar reversal from dimming to brightening in multidecadal observational records around the world (Fig. 1 and figs. S5 to S11). These include the carefully calibrated and maintained sites of the Climate Monitoring and Diagnostics Laboratory (CMDL) (*16*) located in North America (Boulder, Colorado, and Barrow, Alaska), in the North and South Pacific (Mauna Loa, Hawaii, and Samoa), and in Antarctica (South Pole). The CMDL sites show a recent recovery from their downward tendencies before the mid-1980s (Fig. 1 and fig. S5).

The available data in the former Soviet Union allow the analysis of solar radiation up to 1996 for the Moscow region (*17*) (fig. S6). A change from decrease to increase in solar radiation is found during the 1980s, in line with atmospheric transmission measurements in fig. S4. For Japan, the comprehensive data available for the 1990s also suggest a recovery from the prior dimming (Fig. 1 and fig. S7). A strong increase in insolation during the 1990s is apparent in the longest time series available from Japan (Tateno), which goes back to 1958 (fig. S8). This is in line with the increasing atmospheric transmission since the 1980s documented in a unique data set from 14 pyrheliometer sites in Japan (fig. S4). The majority of the sites in China, available in GEBA since 1988, show an increase in insolation as well (Fig. 1 and fig. S9). This supports findings of a recent study based on 85 rural radiation sites in China, where the decline of solar radiation between the 1950s and 1980s levels off in the 1990s [see figure 6 in (*18*)]. A reversal from dimming to brightening during the 1980s is further found at sites in Singapore and Malaysia (Fig. 1 and fig. S10).

A high-quality radiation network was established in 1993 in Australia. Data available up to 2003 do not support a continued dimming, because a majority of the sites show an increase in solar radiation in recent years (Fig. 1 and fig. S11).

Indications of a significant continued dimming in the 1990s are largely restricted to data from India [based on limited data that passed the quality checks (fig. S12)], possibly related

[1]Institute for Atmospheric and Climate Science, Swiss Federal Institute of Technology (ETH), Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. [2]Pacific Northwest National Laboratory, Post Office Box 999, Richland, WA 99352, USA. [3]National Oceanic and Atmospheric Administration Climate Monitoring and Diagnostics Laboratory, 325 Broadway, Boulder, CO 80305, USA. [4]Bureau of Meteorology, Melbourne, Victoria 3001, Australia. [5]Estonian Meteorological and Hydrological Institute, 61602 Toravere, Estonia. [6]Tartu Observatory, 61602 Toravere, Estonia. [7]World Radiation Data Centre, A. I. Voeikov Main Geophysical Observatory, 194021 Saint Petersburg, Russia.

*To whom correspondence should be addressed. E-mail: martin.wild@env.ethz.ch

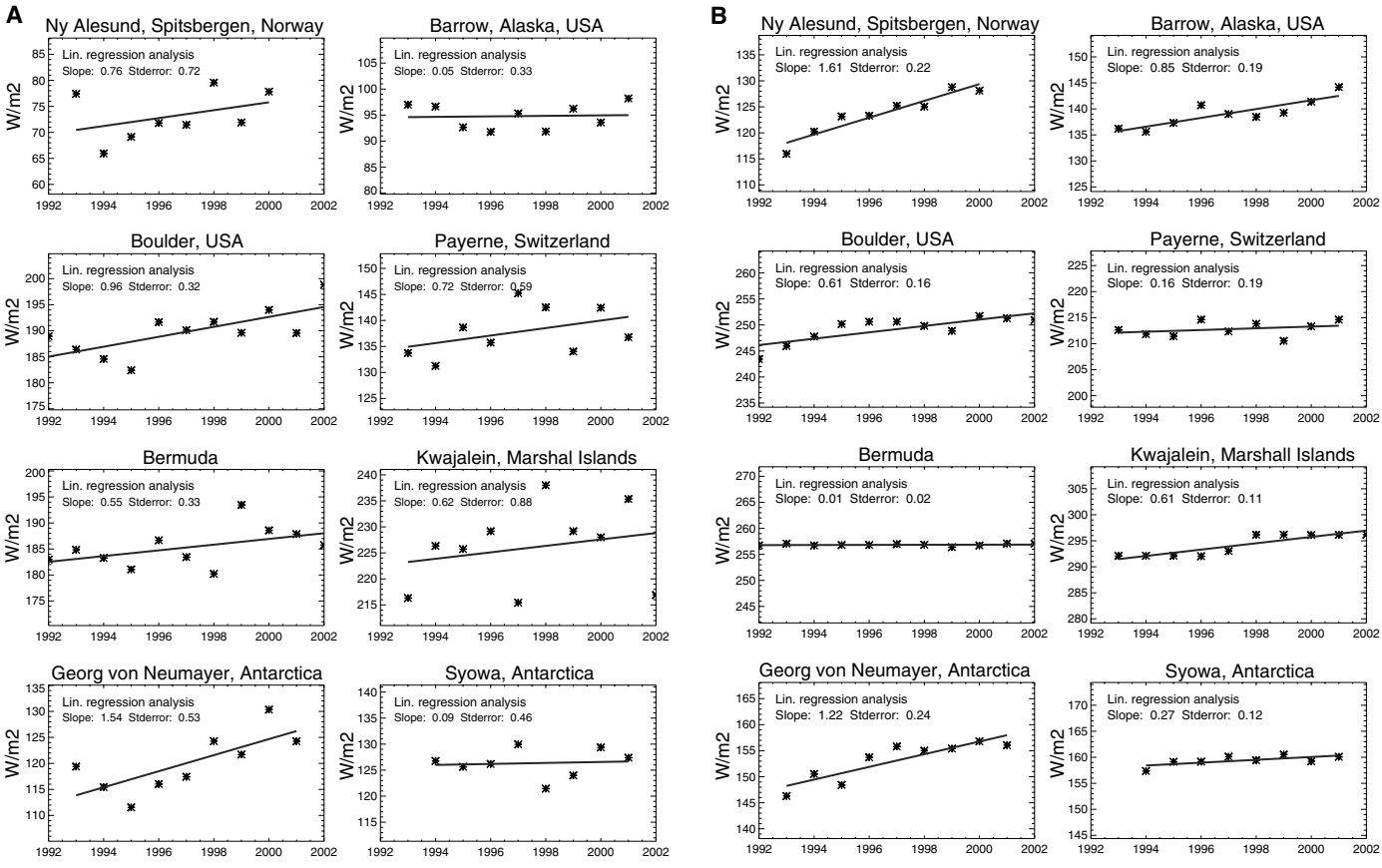

Fig. 2. Time series of annual mean surface solar radiation measured at worldwide distributed sites from BSRN. Shown are the eight longest records from BSRN covering the period 1992 to 2002 for (**A**) all-sky conditions and (**B**) clear-sky conditions (*24*). Solar radiation increases at all sites under both all-sky and clear-sky conditions over this period. Units W m^{-2}.

to the ongoing prevalence of atmospheric brown clouds (ABCs) (*19*). In addition, on the African continent, surface solar radiation decline measured at two sites in Zimbabwe shows no tendency for recovery, whereas the dimming in Egypt levels off during the 1990s (Fig. 1 and figs. S13 and S14).

Data available from various other places do not reach the level of accuracy that would allow for time-series analysis, including sites in South America, Africa, and the United States (during the 1980s), as well as the Australian data prior to 1988; the latter were model-adjusted and thus artificially trend free.

This rather unsatisfactory situation with data quality led to the establishment of BSRN in 1992, where new quality standards for solar radiation measurements were introduced. From BSRN, we selected the sites with the longest available records, including the high-latitude sites Ny Alesund (Spitsbergen, Norway) and Barrow (Alaska, USA) in the Arctic as well as the Georg von Neumayer and Syowa stations in Antarctica, the mid-latitude sites Boulder (Colorado, USA) and Payerne (Switzerland), and the low-latitude sites Bermuda (West Atlantic) and Kwajalein (Tropical West Pacific). Time series of annual mean surface solar radiation at these sites are shown in Fig. 2A with their associated linear fits. It is noteworthy that none of the sites shows a decline. Rather, six of the eight sites show a substantial increase. Similar tendencies are found at other BSRN sites with shorter time series (Fig. 1). Thus, the highest quality data available for the 1990s suggest a brightening rather than a dimming.

Overall, the information contained in the GEBA/WRDC, BSRN, and CMDL records provides no evidence for the continuation of widespread dimming into the 1990s. Instead, there are indications for an increase in surface insolation since the mid-1980s at many locations, mostly in the Northern Hemisphere but also in Australia and Antarctica. A similar reversal to brightening in the 1990s has been found on a global scale in a recent study that estimates surface solar radiation from satellite data (*20*). This indicates that the surface measurements may indeed pick up a large-scale signal. The changes in both satellite-derived and measured surface insolation data are also in line with changes in global cloudiness provided by the International Satellite Cloud Climatology Project (ISCCP) (*21*), which show an increase until the late 1980s and a decrease thereafter, on the order of 5% from the late 1980s to 2002. A recent reconstruction of planetary albedo based on the earthshine method (*22*), which also depends on ISCCP cloud data, reports a similar decrease during the 1990s. Over the period covered so far by BSRN (1992 to 2001), the decrease in earth reflectance corresponds to an increase of 6 W m^{-2} in absorbed solar radiation by the globe (*22*). The overall change observed at the BSRN sites, estimated as an average of the slopes at the sites in Fig. 2A, is 0.66 W m^{-2} per year (6.6 W m^{-2} over the entire BSRN period). The dramatic increase in the planetary albedo estimated in (*22*) for 2002/2003 lies outside the period of available surface measurements and is controversial (*23*).

A further advantage of the BSRN data is their high temporal resolution (minute means), in contrast to conventional radiation data typically available in the form of monthly or daily means. The high-frequency measurements allow a stratification of the BSRN records into cloudy and clear-sky periods on the basis of an advanced clear-sky detection algorithm (*24*). The availability of extended records under both clear- and all-sky conditions for different climatic regimes provides a unique opportunity to study the transmission of solar radiation through the atmosphere. In Fig. 2B, time series of clear-sky insolation aggregated into annual means are shown with their associated linear fits for the eight BSRN sites, with slopes ranging from +0.01 to +1.61 W m^{-2} per year. This suggests that the cloud-free atmosphere

might have become more transparent during the 1990s, in line with atmospheric transmission measurements in fig. S4. During the early 1990s, the increase in atmospheric transmission reflects the recovery from Pinatubo aerosol loadings. In addition, air-quality regulations and the decline of the Eastern European economy may have affected the large-scale aerosol concentration (*25*). The overall increase in the clear-sky fluxes, again estimated as an average over the slopes at the sites in Fig. 2B, is 0.68 W m^{-2} per year, comparable to the increase under all-sky conditions. The similar changes under clear- and all-sky conditions indicate that, besides clouds, changes in the transparency of the cloud-free atmosphere also contributed to the increase in insolation.

To summarize, our data suggest that the widespread decline of solar radiation widely reported for the period of about 1960 to 1990 did not continue in the following years. Rather, there are indications that the amount of sunlight at the surface has increased during the 1990s at most of the locations for which good records exist. This is found under all- and clear-sky conditions, indicating that processes in both cloud-free and cloudy atmospheres contributed to the brightening during the 1990s, possibly pointing to an interplay of direct and indirect aerosol effects.

The absence of dimming since the mid-1980s may profoundly affect surface climate. Whereas the decline in solar energy could have counterbalanced the increase in downwelling longwave energy from the enhanced greenhouse effect before the 1980s (*10*), this masking of the greenhouse effect and related impacts may no longer have been effective thereafter, enabling the greenhouse signals to become more evident during the 1990s.

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Supporting Online Material
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Figs. S1 to S14

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Do Satellites Detect Trends in Surface Solar Radiation?

R. T. Pinker,[1] B. Zhang,[2] E. G. Dutton[3]

Long-term variations in solar radiation at Earth's surface (*S*) can affect our climate, the hydrological cycle, plant photosynthesis, and solar power. Sustained decreases in *S* have been widely reported from about the year 1960 to 1990. Here we present an estimate of global temporal variations in *S* by using the longest available satellite record. We observed an overall increase in *S* from 1983 to 2001 at a rate of 0.16 watts per square meter (0.10%) per year; this change is a combination of a decrease until about 1990, followed by a sustained increase. The global-scale findings are consistent with recent independent satellite observations but differ in sign and magnitude from previously reported ground observations. Unlike ground stations, satellites can uniformly sample the entire globe.

The concept of "global dimming" (*1–3*), which refers to long-term measured decreases in the amount of solar radiation that reaches Earth's surface (*S*), has received prominent attention because of concerns about its possible climatic and environmental implications. An early report on this topic based on surface observations made primarily in Europe (*4*) suggested that *S* declined by more than 10% from 1960 to 1990. On the basis of the analysis of a more comprehensive observational database, it was shown that over land, *S* decreased on the average by 0.23% (*1*) and 0.32% (*2*) per year from 1958 to 1992. The largest decrease was in parts of the former Soviet Union (*5*), where *S* decreased by about 20% between 1960 and 1987. Independent indirect evidence for plausible decreases in *S* has been found in pan evaporation records (*6*, *7*), which show that the rate of evaporation did not increase but rather decreased, in spite of global warming trends evident in records of surface temperatures. When the evaporation data were compared with the global dimming records, the respective tendencies matched, which suggests that these two processes might be linked. Two other studies (*8*, *9*) found that *S* in the Swiss Alps increased between 1995 and 2003 after decreasing from 1981 to 1995 (*8*).

Speculations about possible causes of global dimming include cloud changes, increasing amounts of human-made aerosols, and reduced atmospheric transparency after explosive volcanic eruptions. (Data indicating global dimming could also be produced by instrument deficiencies.) Particles of soot and sulfates absorb and reflect sunlight and facilitate the formation of larger and longer-lasting clouds. The Indian Ocean Experiment (*10*) has clearly documented the large, short-term reduction in solar radiation reaching the surface caused by absorbing aerosols, particularly black carbon and dust. Regionally, the seasonally averaged reduction in the Indian Ocean can reach 10 to 30 W m^{-2}. However, there is some evidence that the reported longer-term

[1]Department of Meteorology, University of Maryland, College Park, MD 20742, USA. [2]Global Modeling and Assimilation Office, NASA Goddard Space Flight Center, Greenbelt, MD 20771, USA. [3]National Oceanic and Atmospheric Administration, Climate Monitoring and Diagnostics Laboratory (NOAA/CMDL), Code R/CMDL1, 325 Broadway, Boulder, CO 80305, USA.

tendencies might not continue, because of the reduction in the levels of air pollution. For example, global dimming over Germany seems to be decreasing, possibly because of a reduction of pollutants (*11*).

Although the potential climatic effects of a sustained decrease or increase in surface solar radiation require extensive investigation of additional factors, such as the long-wave radiative effects of any associated cloud variations, possible effects on photosynthesis are more direct but will differ by location. In equatorial regions that are sufficiently illuminated, photosynthesis is likely to be limited by carbon dioxide or water, not by radiation. It has been reported (*12*, *13*) that in some cases, photosynthesis could increase slightly with a decrease in solar radiation, because the proportion of diffuse light will increase; diffuse light is absorbed less than the direct beam in the external layers of vegetation, and therefore the diffuse light penetrates deeper into the canopy, reaching a larger fraction of the biomass. At high latitudes, plant growth is light-limited, and a decrease in solar radiation can affect net primary productivity.

In this study, the longest available record of satellite-based estimates of surface solar radiation was used to learn about tendencies in this parameter at spatial scales that otherwise would not be possible. The derived fluxes are based on methodologies that have been critically evaluated and that currently serve as a benchmark for deriving surface short-wave radiative fluxes. Specifically, the International Satellite Cloud Climatology Project (ISCCP) (*14*, *15*) data have been used to derive surface radiative fluxes for about 20 years. An extensive effort was undertaken to address the calibration issue of the various satellite sensors used (*16*, *17*), and it is believed that at present, the best attainable calibration techniques were applied. The homogeneity of the satellite observations was achieved by normalizing all of the geostationary satellites used to the polar orbiting satellites, which enjoy a long history of calibration know-how. Specifically, the Satellite Calibration Center (Centre de Meteorologie Spatiale, Lannion, France) receives special high-resolution imaging data from the Satellite Processing Centers that provide data for ISCCP. These data are used to normalize the calibrations of the geostationary satellites to the polar orbiter, which is taken as the standard, and the resulting coefficients are sent to the processing center or the ISCCP data center (http://isccp.giss.nasa.gov/).

The estimated surface-downward solar radiative fluxes were derived with the University of Maryland version of the Global Energy and Water Cycle Experiment (GEWEX) Surface Radiation Budget algorithm (*18*), and the ISCCP D1 satellite data at a spatial resolution of 2.5° was used as input. The monthly mean fluxes were obtained by averaging the daily mean values that were obtained by integration of three hourly instantaneous fluxes. The model allows the computing of upward fluxes at the surface and net solar fluxes at the top of the atmosphere by using satellites observations of clear and cloudy radiances, cloud cover fraction, and independent information on the total amount of water vapor, ozone, and snow cover.

Extensive evaluation of the satellite-estimated monthly mean downward fluxes at the surface against ground measurements has been performed (*19*, *20*). Monthly averaged, hemispheric, broadband, solar-surface measurements from the World Radiation Data Center, the Global Energy Budget Archive, and the Canadian Network were used. These measurements provide a continuous, long, time series that is clustered in regions with similar climatological surface types (such as Europe, southeast Africa, East Africa, and south Australia) that are used in the evaluation. The range of the root mean square (RMS) errors at a spatial resolution of 2.5° was found to be between 11.7 and 31.5 W m^{-2} (*19*); the best results were found over Europe, Australia, eastern Canada, and western Canada (known for good quality of ground data); the worst were found over central Africa and Pakistan/India. In an updated evaluation at 1° resolution based on ISCCP DX data (*20*, *21*), the range of the RMS errors was reduced to about 7.5 to 27.0 W m^{-2} (excluding African stations). On the basis of results of evaluation against Surface Radiation (SURFRAD) (*22*) ground observations at a spatial resolution of 0.5° over the United States, it was found that the average RMS error on a monthly time scale was about 20 W m^{-2} (*23*).

Two independent approaches were used to determine tendencies of the data: linear and second-order fits. The confidence levels of the derived tendencies were calculated according to the Student's *t*-test distribution with $n - 2$ degrees of freedom

$$t = r_{xy}\sqrt{\frac{n-2}{1-r_{xy}^2}}$$

Here r_{xy} is the correlation coefficient between the original time series and the linear and second-order fitted time series (*24*), and n is the number of observations.

Figure 1 presents linear and second-order least-squares fits to the original satellite-derived time series of surface solar irradiance (1983 to 2001) after removal of the mean annual cycle. On a global scale, the linear slope (solid line) in the surface solar radiation is positive at 0.16 W m^{-2} year^{-1}. The second-order polynomial (broken line) indicates a small decrease during the time period from 1983 to 1992, with a reversal around 1992. Both the linear and the second-order fits are significant at a 99% level of confidence.

Results from similar analyses are presented in Fig. 2, which were computed from the following: satellite-based observations over the Arctic (60° to 90°N) (Fig. 2A); satellite-based observations over a local point in the Arctic (Barrow, Alaska) (Fig. 2B); and ground observations at Barrow, Alaska (Fig. 2C). To be consistent with the analysis for American Samoa (Fig. 3), adjacent satellite grid points were averaged over the ground location for Fig. 2B. The ground observations are from the National Oceanic and Atmospheric Administration, Climate Monitoring and Diagnostics Laboratory (NOAA/CMDL) (*25*) and are considered to be the highest quality available for long-term ground observations. Analysis of the satellite-based observations shows a small decrease in solar radiation over the 60° to 90°N Arctic region from 1983 to 2001. The linear slopes for all three cases in Fig. 2 are slightly negative. However, the second-order fits show a decrease in solar radiation from 1983 to about 1992 to 1993 that is followed by a reversal in tendency. This is the first time that consistency in long-term temporal variations between satellite observations and ground observations has been documented. These results also indicate that the radiative conditions at the Barrow site behave similarly to those over the latitudinal belt of 60° to 90°N. The results are consistent with independent findings that report warming in the Arctic from 1982 to 1999 and an increase in

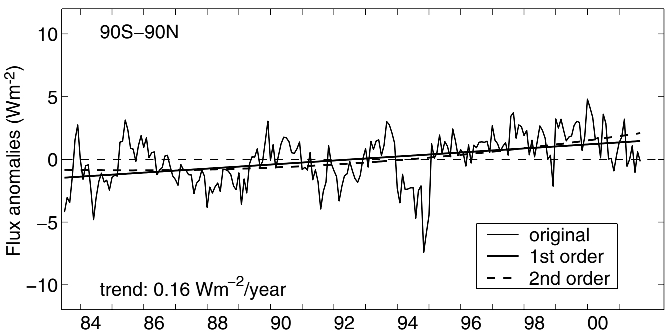

Fig. 1. Linear and second-order least-squares fits to the original satellite-derived time series of *S* (from 1983 to 2001) averaged over the globe, after removal of the mean annual cycle. The linear slope (solid line) of the surface solar radiation is positive at 0.16 W m^{-2} year^{-1}. The second-order polynomial (dashed line) indicates a small decrease from 1983 to 1992, with a reversal around 1992. Both the linear and the 2nd-order fits are significant at the 99% level of confidence.

cloudiness during the spring and summer (*26*), when most of the radiation in this region is received. Correlations between the actual ground observations at Barrow with the satellite estimates were computed and found to be 0.97 (fig. S1). A similar experiment has been performed at American Samoa, a site where high-quality observations are available (*25*) and which represents oceanic conditions (Fig. 3). Observed tendencies from satellite estimates of the surface solar fluxes are shown (Fig. 3A), and Fig. 3B represents the same, using the CMDL ground observations. Because local cloud effects caused by land/water boundaries affect the observations at the Samoa site, the satellite estimates were averaged over grid cells adjacent to the one centered over the ground site. Here, the linear slopes are positive and the second-order fit indicates a decrease in radiation from 1983 to about 1992 to 1993 that is followed by an increase thereafter. Correlations between the actual ground observations at this site with the satellite estimates were also computed and found to be 0.86 (fig. S1). We investigated the tendencies of the surface solar radiation in the tropical belt of 20°S to 20°N (Fig. 4A) and at the top of the atmosphere (ToA) (Fig. 4B). It was found that at the surface, there is a positive linear increase of about 0.18 W m^{-2} $year^{-1}$, which indicates an increase in the surface radiation. At the ToA, the situation is reversed and the decease is about –0.17 W m^{-2} $year^{-1}$. The tendencies from the second-order fit are similar to the linear ones. A decreasing tendency is also reported at the ToA's reflected solar radiation (*27*), which is observed by a combined data set based on observations from the Clouds and the Earth's Radiant Energy System (CERES) (*28*) and from the Earth Radiation Budget Satellite (ERBE) (*29*). It is claimed that the observed changes in radiation budget are caused by changes in the mean tropical cloudiness, which is detected in the satellite observations but fails to be predicted by several current climate models.

The studies that reported dimming were conducted over land; therefore, a separate analysis of tendencies over the land and oceans was performed (Fig. 5). A land mask as described in (*30*) was used. We considered sea ice–covered oceans as ocean. The tendencies over land global domain from 90°S to 90°N have been found to be slightly negative at about –0.05 W m^{-2} $year^{-1}$, and over the oceans, the tendency was positive at 0.24 W m^{-2} $year^{-1}$. For the

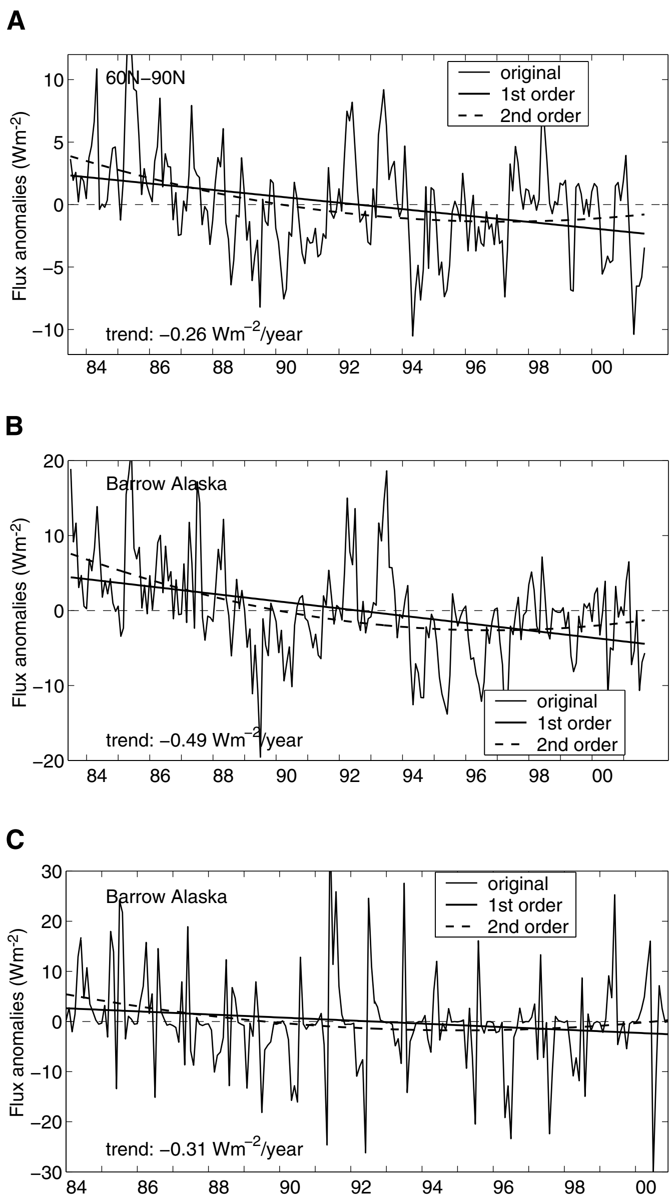

Fig. 2. Linear and second-order least-squares fits over (**A**) the Arctic 60° to 90°N, (**B**) over Barrow Alaska from satellites, and (**C**) at Barrow from ground observations.

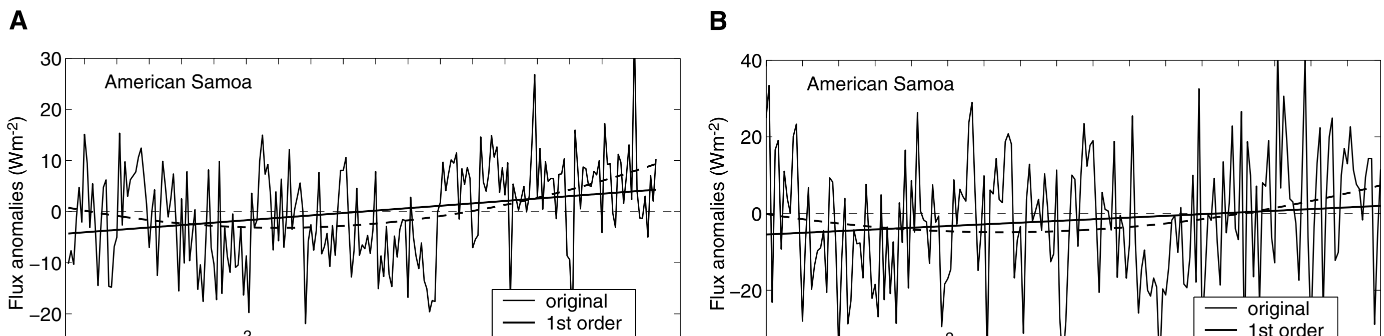

Fig. 3. Linear and second-order least-squares fits over (**A**) American Samoa from satellites and (**B**) at American Samoa from ground observations.

land case, the trend is not statistically significant; however, for the ocean, it is significant with a confidence level of 99%. Because long-term ground observations are available mostly from land sites, it is possible that land-based observations are not representative of Earth as a whole, and therefore brightening at a global scale is possible. For the land-alone case, although the overall slope is negative, there is a small increase after 1993.

About 20 years (from 1983 to 2001) of *S* fluxes at Earth's surface that were derived from satellite observations were analyzed. (The annual mean surface solar radiation in W/m^2 from 1983 to 2001, as distributed over the globe at 2.5° spatial resolution estimated from the ISCCP D1 data, is illustrated in fig. S2.) The analysis was conducted at a global scale as well as over regions of special climatic significance. Our findings at the global scale as well as those in the tropical belt and in the Arctic are consistent with the findings of independent satellite studies (*26*–*28*). For example, a global-scale decrease in cloudiness (*31*) based on ISCCP D1 data was found, which is consistent with an increase in surface solar radiation found in this study, because clouds are the major modulators of the solar radiation that reaches the surface. Warming in the Arctic from1982 to 1999 and an increase in cloudiness during the spring and summer (*26*) is reported, which is also consistent with our findings in this region. Moreover, since the mid-1960s, the melt date in northern Alaska has advanced by 8 days (*32*) as a result of a decrease in snowfall in winter, followed by a warmer spring, which are believed to be caused by variations in regional circulation patterns. A significant variation in photosynthetic activity and growing season length at latitudes above 35°N from 1982 to 1999 was also reported, which is indicated by the Normalized Difference Vegetation Index (NDVI) derived from the Advanced Very-High Resolution Radiometer (AVHHR) onboard the polar-orbiting NOAA meteorological satellites (*33*). Two distinct periods of increasing plant growth are apparent: 1982 to 1991 and 1992 to 1999, which are separated by a reduction from 1991 to 1992 that is associated with the volcanic eruption of Mt. Pinatubo in June 1991. The average May to September NDVI from 45° to 75°N increased by 9% from 1982 to 1991, decreased by 5% from 1991 to 1992, and increased by 8% from 1992 to 1999. In an independent study based on the AVHRR data for the period from 1982 to 2000. it was found that in the Northern Hemisphere, the area with an increasing trend of the annual sum of NDVI was approximately 12 times larger than the area with a decreasing trend. Although these areas are located over a large range of geographical regions, they include Siberia, northeastern Europe, and the northern part of North America (*34*). At high latitudes, plant growth is light-limited, and therefore a decrease in solar radiation would not be conducive to an increase in the vegetation index. On the basis of on observations made from CERES (*28*) and a 16-year record from the ERBS mission (*29*), a decrease in reflected short-wave flux at the ToA (*27*) was found. This could result in an increase in *S* (assuming no variations in the solar output or in atmospheric absorption).

On the basis of earthshine measurements (*35*) of Earth's reflectance carried out at the Big Bear Solar Observatory since 1998 and satellite observations of global cloud properties for earlier years, a proxy measure of Earth's global short-wave reflectance was constructed. A steady decrease in Earth's reflectance from 1984 to 2000 was shown, with a strong drop during the 1990s. During 2001 to 2003, only earthshine data are available, and they indicate a reversal of the decline. It should be noted that the earthshine measurements are available for only a short time period, and the extension to a longer period is achieved by using ISCCP data.

We report here on an attempt to use long-term satellite observations as obtained under the World Climate Research Programme GEWEX ISCCP initiative to study possible trends in the *S*. Averaged over the entire period of available record and at a global scale, a small increase in *S* was observed rather than

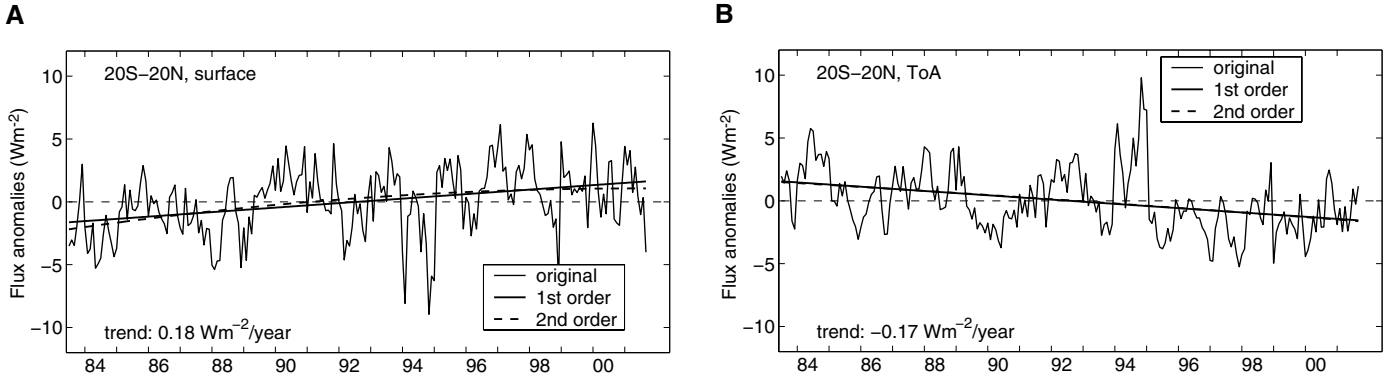

Fig. 4. Linear and second-order least-squares fits over (**A**) the tropical belt of 20°S to 20°N at the surface and (**B**) at ToA.

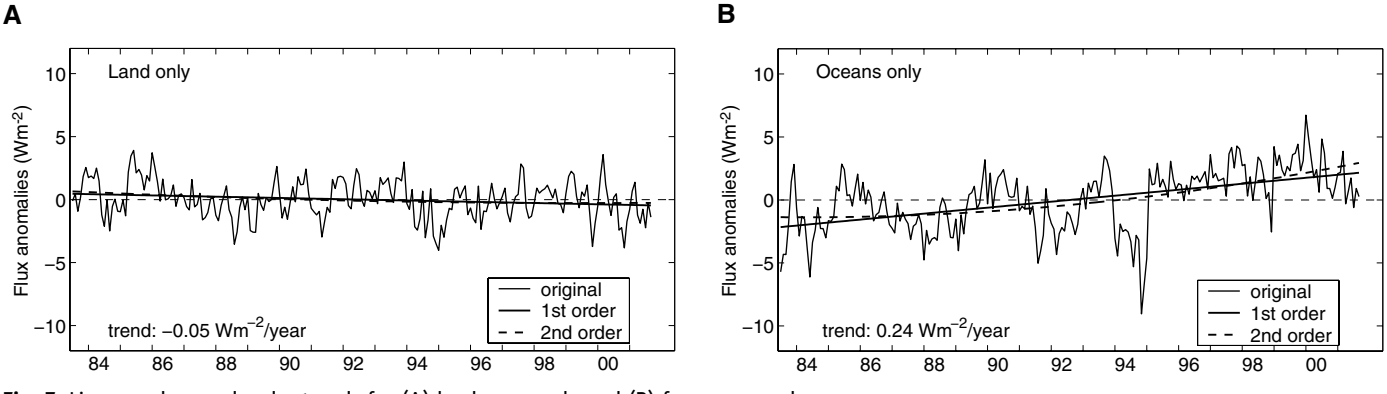

Fig. 5. Linear and second-order trends for (**A**) land areas only and (**B**) for oceans only.

a dimming. This increase has been found to be significant at the 99% level of confidence. The satellite-based record of surface solar fluxes from 1983 until 1992 does suggest some dimming, followed by an increase after 1992, as seen in numerous ground observations. It was also shown that tendencies over land and over ocean can differ in sign and magnitude, and that in order to obtain a global view of the dimming phenomena, there is a need for comprehensive and global observations that are possible only from satellites. There is a need to be aware of calibration issues regarding both ground-based and satellite data that might affect the interpretation of long-term observations. The best available approach to calibration was used to produce the satellite observations used in this study, and the most comprehensive global coverage achievable by combining geostationary and polar-orbiting satellites was used. The magnitudes of the observed tendencies in *S* at a global scale were much smaller in magnitude than those reported from ground observations.

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The Holocene Asian Monsoon: Links to Solar Changes and North Atlantic Climate

Yongjin Wang,[1] Hai Cheng,[1,2]* R. Lawrence Edwards,[2] Yaoqi He,[1] Xinggong Kong,[1] Zhisheng An,[3] Jiangying Wu,[1] Megan J. Kelly,[2] Carolyn A. Dykoski,[2] Xiangdong Li[4]

A 5-year-resolution absolute-dated oxygen isotope record from Dongge Cave, southern China, provides a continuous history of the Asian monsoon over the past 9000 years. Although the record broadly follows summer insolation, it is punctuated by eight weak monsoon events lasting ∼1 to 5 centuries. One correlates with the "8200-year" event, another with the collapse of the Chinese Neolithic culture, and most with North Atlantic ice-rafting events. Cross-correlation of the decadal- to centennial-scale monsoon record with the atmospheric carbon-14 record shows that some, but not all, of the monsoon variability at these frequencies results from changes in solar output.

The impacts of decadal- to centennial-scale solar variability on the climate system during the Holocene have been reported from mid to high northern latitudes (*1*–*3*) to low-latitude regimes (*4*–*6*), including the Asian monsoon (AM) (*4*, *5*). To test the degree to which the Holocene AM may be linked to solar variability, a high-resolution, precisely dated, continuous record of the monsoon is needed. Such a record could also be used to test the degree to which changes in the interglacial AM are related to climate change elsewhere. For example, a number of studies have demonstrated close ties between the glacial AM and the climate in the North Atlantic region (*7*–*9*). The degree to which such links extend into interglacial periods is an open question. We have previously reported on a Chinese Holocene record of the AM that addresses some of these issues (*10*). Here, we build on that work with a higher resolution absolute-dated Holocene AM record from Dongge Cave, southern China, which we compare in detail with the atmospheric ^{14}C record (as a proxy for solar output) and climate records from the North Atlantic region (*2*, *11*–*13*).

Stalagmite DA was collected from Dongge Cave (25°17′N, 108°5′E, elevation 680 m) in southern China. Today, the cave site has two distinct seasons: a cool, dry season during the boreal winter when the Siberian high establishes a strong anticyclone on the Tibetan Plateau and a warm, wet season during the summer months when the intertropical convergence zone (ITCZ) shifts northward and monsoonal convective rainfall reaches its maximum. Our previous studies have shown that shifts in the oxygen isotope ratio ($\delta^{18}O$) of the stalagmite from the cave largely reflect changes in $\delta^{18}O$ values of meteoric precipitation at the site, which in turn relate to changes in the amount of precipitation and thus characterize the AM strength (*10*, *14*).

Chronology of the 962.5-mm-long stalagmite DA is established by 45 ^{230}Th dates (table S1), all in stratigraphic order, with a typical age uncertainty of 50 years. Sample DA grew continuously from approximately 9000 years before the present (ky B.P., where the "present" is defined as the year 1950 A.D.) until 2002 (when the stalagmite was collected), with a nearly constant growth rate of ∼100 μm per year. A total of 2124 $\delta^{18}O$ measurements were obtained

[1]College of Geography Science, Nanjing Normal University, Nanjing 210097, China. [2]Department of Geology and Geophysics, University of Minnesota, MN 55455, USA. [3]State Key Lab of Loess and Quaternary Geology, Institute of Earth Environment, Chinese Academy of Sciences, Xi'an 71005, China. [4]Department of Civil and Structural Engineering, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong.

*To whom correspondence should be addressed. E-mail: cheng021@umn.edu

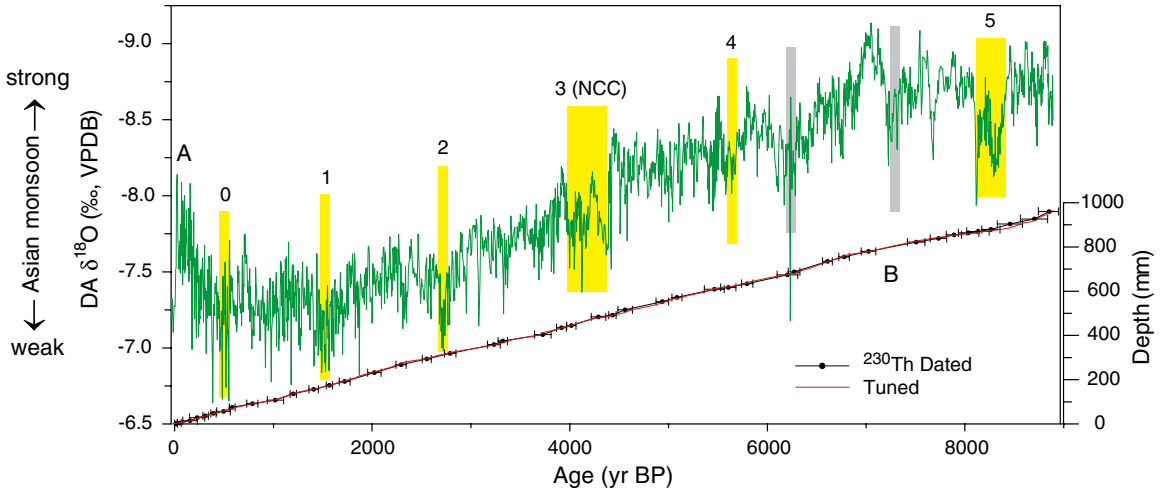

Fig. 1. (**A**) $\delta^{18}O$ time series of the Dongge Cave stalagmite DA (green line). Six vertical yellow bars denote the timing of Bond events 0 to 5 in the North Atlantic (*2*). The Chinese events that correlate with Bond events 3 and 5 coincide within error with the collapse of the Neolithic Culture of China (NCC) (*23*) and the timing of an abrupt outflow event from a Laurentide ice-margin lake (*22*), respectively. Two vertical gray bars indicate two other notable weak AM events that can be correlated to ice-rafted debris events (*2*). (**B**) DA age-depth (mm, relative to the top) relations. Black error bars show ^{230}Th dates with 2σ errors (table S1). We use two different age-depth curves, one employing linear interpolation between dated depths and the second slightly modified by tuning to INTCAL98 (*15*) within the ^{230}Th dating error (*26*).

from along the growth axis, with an average temporal resolution of 4.5 years (table S2). We present two time scales, one of which is based on linear interpolation between the ^{230}Th dates and is independent from all of the chronologies to which we compare our data (Fig. 1). Another is tuned within dating error to INTCAL98 (*15*) (Fig. 1) and is used to determine the extent to which fine-scale dating errors may affect our correlations with other records.

The $\delta^{18}O$ values vary between –9.2 and –6.5 ‰, with a typical amplitude of somewhat less than 1 ‰ over time scales of decades to centuries. As verified by our previous studies (*10*, *14*), Dongge Cave $\delta^{18}O$ becomes lower as Asian summer monsoon intensifies, and vice versa. Such anticorrelation is also observed in the modern precipitation records near the cave site (*16*).

DA $\delta^{18}O$ data shows a strong AM interval from 9 to 7 ky B.P., followed by a gradual weakening. This overall temporal pattern resembles high-resolution Holocene precipitation records from a southern Oman stalagmite (*5*), titanium concentration data from the Cariaco Basin, tropical Atlantic (*17*), and our earlier work (*10*), which suggests that shifts in mean position of the ITCZ may control temporal variability of precipitation throughout the entire low-latitude region (*18*). This general weakening of the Asian monsoon during the Holocene corresponds with orbitally induced lowering of Northern Hemisphere summer solar insolation during this interval, which indicates that the broad trend is caused by insolation change. The general trend is punctuated by eight weak AM events, each lasting ~1 to 5 centuries, centered at 0.5, 1.6, 2.7, 4.4, 5.5, 6.3, 7.2, and 8.3 ky B.P., with a temporal spacing averaging ~1.2 ky (Fig. 1). These events are, within dating error, in phase with weak southwest AM events recorded in marine cores from the Arabian Sea (*19*) and are possibly linked to Holocene ice-rafting events in the North Atlantic (*2*) (Fig. 1). Among those events, the two at 8.4 to 8.1 and 4.5 to 4.0 ky B.P. are longer in duration and larger in magnitude. They are similar in terms of the abrupt transitions and magnitude (0.8 to 1.0 ‰), and they have one or two brief reversions within the events. The event at 8.4 to 8.1 ky B.P. correlates with the strongest Holocene cooling/drying event recorded at high northern latitudes (*1*, *20*) and subtropical temperate regions (*21*) and in tropical ocean and terrestrial records (*4*, *5*, *19*); it also coincides within error with the 8.2-ky event recorded in the Greenland ice cores (*11*, *12*), possibly related to abrupt outflow from a Laurentide ice-margin lake (*22*). Another major event at ~4.4 to 3.9 ky B.P., although not clear in Greenland records, has been reported in various localities in China (*23*). Among the most abrupt events in the Holocene Dongge record is the abrupt lowering of AM intensity at ~4.4 ky B.P. over several decades (Fig. 1 and table S2), which supports the idea that this sharp hydrological change might be responsible for the collapse of the Neolithic culture around Central China about 4.0 ky ago (*23*). Strongly enhanced aridity at this time is also a main feature of the Indian monsoon as recorded in western China (*24*) and is in phase with the Mesopotamian dry event in western Asia (*25*).

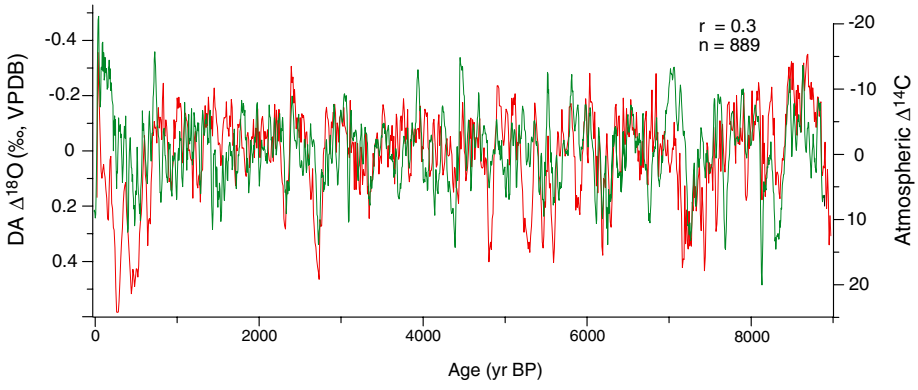

Fig. 2. Time series of the DA $\Delta^{18}O$ record (five-point running average, green line) and the atmospheric $\Delta^{14}C$ record (red line) (*15*). All data have been detrended using singular spectrum analysis. Higher solar irradiance (smaller $\Delta^{14}C$) corresponds to a stronger AM (smaller $\Delta\delta^{18}O$ value). The correlation coefficient is 0.30 for the entire profile and 0.39 between 9 and 6 ky B.P.

To assess the link between solar activity and AM intensity, we compared the detrended DA $\delta^{18}O$ ($\Delta^{18}O$) record to the detrended atmospheric ^{14}C record ($\Delta^{14}C$), a proxy for solar activity (*15*) (Fig. 2), using the tuned time scale for DA (*26*). As the time scale is tuned, we consider the resulting correlation to be a "best case" scenario. Visually, (Fig. 2) the larger amplitude fluctuations in the AM (>±0.2 ‰ in the $\Delta\delta^{18}O$ record) broadly agree with $\Delta^{14}C$ events on centennial time scales, similar to the relation observed in the record from a southern Oman stalagmite (*5*). The correlation coefficient for the full record is $r = 0.30$, which indicates that some of the variability in the AM can be attributed to solar changes. The main discrepancy between the two records comes at decadal time scales, plausibly reflecting fine-scale errors in chronolo-

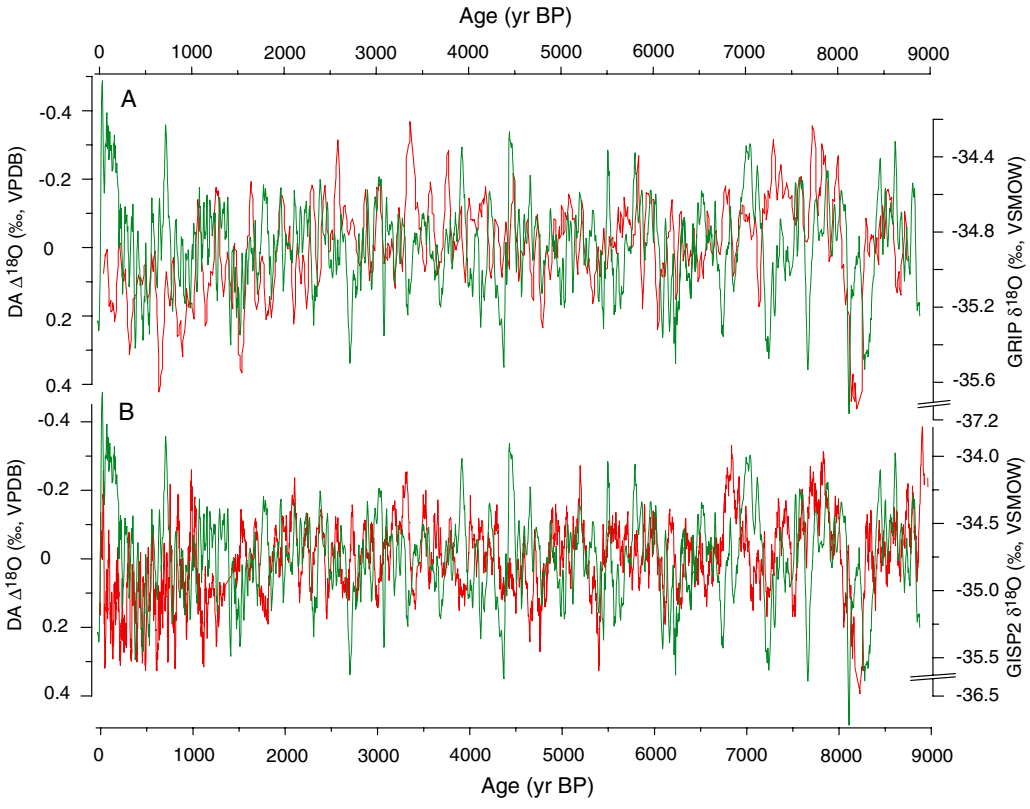

Fig. 3. Comparison of the smoothed (5-point running average) detrended DA $\Delta^{18}O$ record (green) with the smoothed 20-year averaged GRIP $\delta^{18}O$ record (5-point running average, red) (*11*) (**A**) and the GISP2 $\delta^{18}O$ record (20-point running average, red) (*12*) (**B**) over the past 9 ky. The broad correlations between DA and Greenland records are apparent at the multicentennial scale.

gy or, alternately, indicating that at these frequencies other factors may be more important in controlling AM variability, such as changes in atmospheric and oceanic circulation.

Power spectral analysis of the tuned DA $\delta^{18}O$ record shows statistically significant centennial periodicities centered on 558, 206, and 159 years (fig. S1A). These periodicities are close to significant periods of the $\Delta^{14}C$ record (512, 206, and 148 years) (*27*) and to previously reported findings from spectral analysis of another Chinese speleothem (*10*). Cross-spectral analysis of the DA record and the ^{14}C record further shows some common periodicities (232, 129, 116, 104, 89, 57, and 54 years) (fig. S1B). Our data, together with the other Chinese work (*10*) and two Oman stalagmite $\delta^{18}O$ records (*4*, *5*), support the idea that solar changes are partly responsible for changes in Holocene AM intensity (*28*).

We have previously demonstrated a close correlation between last glacial period AM variability and the temperature change over Greenland on millennial time scales (*9*, *14*). The present high-resolution DA $\delta^{18}O$ record enables a more precise correlation between the AM and Greenland climate on centennial time scales and under interglacial conditions. The smoothed, detrended DA $\Delta^{18}O$ record shows a broad similarity to the $\delta^{18}O$ records of Greenland ice—Greenland Ice Core Project (GRIP) (*11*) and Greenland Ice Sheet Project 2 (GISP2) (*12*)—in terms of frequent decadal-scale and centennial-scale fluctuations (Fig. 3). Similar to Greenland ice core records, the centennial- and multidecadal-scale AM variations during the Holocene are considerable (~0.2 to 0.7 ‰ in $\delta^{18}O$) but not as large as glacial millennial-scale variability (~1 to 2 ‰ in $\delta^{18}O$) (*9*). Because of fine-scale uncertainties in dating of records from both sites, it is not possible to determine whether decadal-scale variations correlate. However, the general correlations between DA and Greenland records are apparent on the multicentennial scale (Fig. 3). This broad correlation is also noticeable between DA and the new $\delta^{18}O$ record of Greenland ice [NGRIP (*13*)], which also has a long-term trend similar to DA between 0 and 3.8 ky B.P. (fig. S2). Over this time interval, the Pearson correlation coefficient of the records reaches its highest value of 0.57 when setting a 150-year-phase lead of the tuned DA $\delta^{18}O$ record over the NGRIP $\delta^{18}O$ time series (fig. S2). A lead of this magnitude in this time interval would be larger than the combined uncertainty in the DA ^{230}Th dating and the Greenland layer-counting chronology, but not by a large amount, because both records have errors of up to several decades. If the lead is real, given that we can attribute at least some of the variability in the AM to solar changes, it is plausible that the AM responds almost immediately to solar changes by rapid atmospheric response to solar forcing. Because Greenland's climate is closely tied to the rate of production of North Atlantic Deep Water, it is plausible that Greenland temperature lags solar forcing because of the time constants involved in changing ocean circulation. Alternatively, it is plausible that the apparent lead is not a "true" lead and that the high Pearson correlation coefficient is simply, by chance, higher with a 150-year offset. We note that the DA record has significant power at both 159-year and 206-year periods (fig. S1). Thus, the lead could plausibly represent an offset of one period at one of these frequencies.

In summary, the broad decline in AM intensity through the latter part of the Holocene correlates well with other northern low-latitude records and results directly from the orbitally induced lowering of summer insolation affecting ITCZ position and low-latitude precipitation patterns. The centennial- and multidecadal-scale events that characterize the AM record throughout can, in part, be ascribed to responses to changes in solar output. There are similarities and correlations between the Holocene AM and the North Atlantic climate, including both the ice-rafted debris record and the Greenland ice core records. Some of these correlations result from solar forcing affecting climate in both regions. It is also possible, with the 8200-year event as the main example, that oceanic circulation changes in the North Atlantic triggered changes in the AM. Thus, changes in the Holocene AM result from a number of factors, including orbitally induced insolation changes, changes in solar output, and changes in oceanic and atmospheric circulation.

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Computational Thermostabilization of an Enzyme

Aaron Korkegian,[1,2] Margaret E. Black,[4] David Baker,[3] Barry L. Stoddard[1]*

Thermostabilizing an enzyme while maintaining its activity for industrial or biomedical applications can be difficult with traditional selection methods. We describe a rapid computational approach that identified three mutations within a model enzyme that produced a 10°C increase in apparent melting temperature T_m and a 30-fold increase in half-life at 50°C, with no reduction in catalytic efficiency. The effects of the mutations were synergistic, giving an increase in excess of the sum of their individual effects. The redesigned enzyme induced an increased, temperature-dependent bacterial growth rate under conditions that required its activity, thereby coupling molecular and metabolic engineering.

Enzymes are the most efficient catalysts of chemical reactions known, enhancing reaction rates by as much as 23 orders of magnitude (*1*, *2*). However, there has been little evolutionary pressure for them to become more thermostable than is required by their native environment. Many studies indicate that enzymes (like most proteins) exhibit closely balanced free energy profiles for folding and unfolding, thereby allowing functionally important dynamic motions and appropriate degradation in vivo (*3*). However, in a laboratory or industrial setting, this lack of thermostability can lead to undesirable loss of activity (*4*).

The physical principles of protein folding that result in a balance of stability and flexibility, while maintaining function, are not perfectly understood and have been difficult to exploit for the development of thermostabilized enzymes (*4*). For hyperthermophiles, selective pressures have generated proteins with denaturation temperatures upwards of 110°C (*5*). Their proteins exhibit topologies and stabilizing interactions similar to those from mesophilic and thermophilic organisms (*6*, *7*), leading to diverse hypotheses regarding their relative behaviors (*8*). However, a key mechanism for thermostabilization appears to be the optimization of interactions between amino acids within a protein's core (*5*), complementing computational design methods that optimize a sequence for a given fold (*9–13*).

The thermostabilization of an enzyme presents additional challenges for computational protein design methods, because the active-site substrate geometry and the molecular dynamic behavior during an enzymatic reaction often appear fine-tuned for maximum catalytic efficiency (*2*, *3*). Therefore, the design method must be capable of predicting thermostabilizing mutations within a given fold while minimizing any shift in the backbone that might structurally disrupt the active-site structure or quench its flexibility.

In the past several years, methods for computational protein structure prediction and design have improved substantially (*10*, *11*, *14*). Recently, computational design has been used successfully in thermostabilizing noncatalytic proteins (*15–18*), redesigning binding pockets (*19–23*), creating a protein fold (*24*), and designing catalytic activity into a bacterial receptor (*25*). We use the program RosettaDesign (*26*), which uses an energy function for evaluating the fitness of a particular sequence for a given fold and a Metropolis Monte Carlo search algorithm for sampling sequence space. The program requires a backbone structure as input and generates sequences that have the lowest energy for that fold.

We picked the homodimeric hydrolase enzyme yeast cytosine deaminase (yCD), which converts cytosine to uracil, as a target for computational thermostabilization. yCD was chosen because its high-resolution crystal structure is available (*27*), its catalytic mechanism is well characterized (*27*), it is thermolabile (*28*, *29*), and it has potential use in antitumor suicide gene applications (*27*, *29–31*). As do many commercially useful enzymes, yCD displays irreversible unfolding behavior at high temperatures (presumably because of aggregation) rather than the more simple, fully reversible behavior common among model systems for the study of protein folding. The problems inherent in engineering such catalysts have been recently reviewed (*4*). We used computational redesign to predict a series of point mutations in the enzyme core that might lead to thermostabilization of the enzyme without losing catalytic efficiency. We then prepared a series of designed enzyme variants and determined their folded thermostability, catalytic behavior, ability to complement metabolic cytosine deaminase activity, and three-dimensional crystal structures.

Our general computational strategy was largely unchanged from that described by Kuhlman and Baker (*26*, *32*). An energy function evaluated target sequences threaded onto a template backbone (*12*, *13*, *26*, *33*). Sequence space was searched with an iterative Metropolis Monte Carlo procedure, starting with a random sequence, replacing a single amino acid rotamer with a rotamer from the Dunbrack backbone-dependent rotamer library (*34*), and reevaluating the energy. Sequences with lower energy were automatically adopted, whereas

[1]Division of Basic Sciences, Fred Hutchinson Cancer Research Center (FHCRC), 1100 Fairview Avenue North, Seattle, WA 98109, USA. [2]Graduate Program in Molecular and Cellular Biology, [3]Department of Biochemistry and Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195, USA. [4]Department of Pharmaceutical Sciences, College of Pharmacy, Washington State University, Pullman, WA 99164–6534, USA.

*To whom correspondence should be addressed. E-mail: bstoddar@fhcrc.org

sequences with higher energy were accepted with a probability based on the Metropolis criterion in order to prevent trapping in a local energy minimum.

All residues directly involved in catalysis, those located within 4 Å of the active site, and those involved in the dimer interface were held fixed (fig. S1). The remaining 65 residues of the 153-residue monomer were included in the redesign, allowing them to be changed to any amino acid except cysteine. Thirty-three of the 65 residues subjected to redesign (49%) remained wild-type, a result similar to those of prior applications (*18*, *26*). Sixteen of the point mutations suggested by the program were located on the surface of the protein and were not pursued, whereas the remainder were in the core. The core mutations could be further subdivided into two localized clusters of interacting residues, as well as four additional isolated point mutations (table S1) (*22*).

Site-directed mutagenesis was used to generate each of the two complete clusters of point mutations and the four individual mutations described above. Cluster 1, consisting of nine simultaneous mutations packed between an α helix and several β-strands (including replacement of a buried salt-bridge), aggregated at concentrations above 0.4 mg/mL and was not characterized further. Cluster 2, consisting of four mutations packed between two α helices, remained soluble when concentrated to 20 mg/mL. Individual mutations from this cluster revealed that A23L and I140L (*35*) were key to the thermostabilization of the enzyme and were included in the final construct described below. Of the remaining four individual mutations, one (V108I) was also incorporated in the thermostabilized triple-mutant enzyme, whereas the remaining three (W10T, T67E, and E69L) were not as well behaved and were not characterized further. Both the double mutant (A23L/I140L) and the final triple mutant (A23L/I140L/V108I) were well behaved during expression and purification, more thermostable than the wild-type enzyme, and fully active (table S1) (*22*).

We performed thermal denaturation experiments on all constructs using circular dichroism (CD) spectroscopy (Fig. 1A). Wild-type yCD and the redesigned mutants displayed largely reversible unfolding behavior over the range of temperatures examined; however, at higher temperatures, they unfolded irreversibly (*36*). We quantified the thermal stability of yCD and the mutant constructs by deriving an apparent melting temperature (T_m) from the CD-unfolding curves. This value for the wild-type enzyme was determined to be 52°C. The isolated single mutations A23L, I140L, and V108I each slightly thermostabilized the enzyme, increasing the apparent T_m by ~2°C. However, simultaneous incorporation of all three mutations increased apparent T_m to 62°C, 10°C higher than that of the wild type. Therefore, combination of individual point mutations in a single construct produced a synergistic effect beyond their individual contributions. This result is not simply due to the formation of contacts between redesigned residues, because residue 108 was physically separated from residues 23 and 140.

The kinetic behavior of the wild-type enzyme and the double and triple mutants was measured at 22°C to determine the effects of the mutations (Table 1 and fig. S2), as were their relative activities as a function of temperature (fig. S3). At 22°C, the wild-type enzyme displays a turnover (k_{cat}) of 160 mol (mol enzyme)$^{-1}$ s^{-1} and a Michaelis constant K_m of 1.98 mM, and the double and triple mutants displayed a slightly reduced maximum rate V_{max} coupled with a reduction in the K_m. The catalytic efficiency of the enzyme mutants (expressed as the ratio k_{cat}/K_m) was unchanged relative to the wild-type enzyme. The overall temperature activity profile was broadened, for the redesigned enzyme, with near–wild-type activity retained at lower temperatures and higher activity above 50°C.

The preservation of overall catalytic efficiency (achieved by reducing both k_{cat} and K_m, rather than by maximizing overall velocity) and the unusual change in shape and breadth of the enzyme's thermal profile might suggest that the computational redesign generated mutations that natural or directed evolution pathways might not select, except perhaps as intermediate species. Therefore, computational strategies for thermostabilization might offer a bonus of

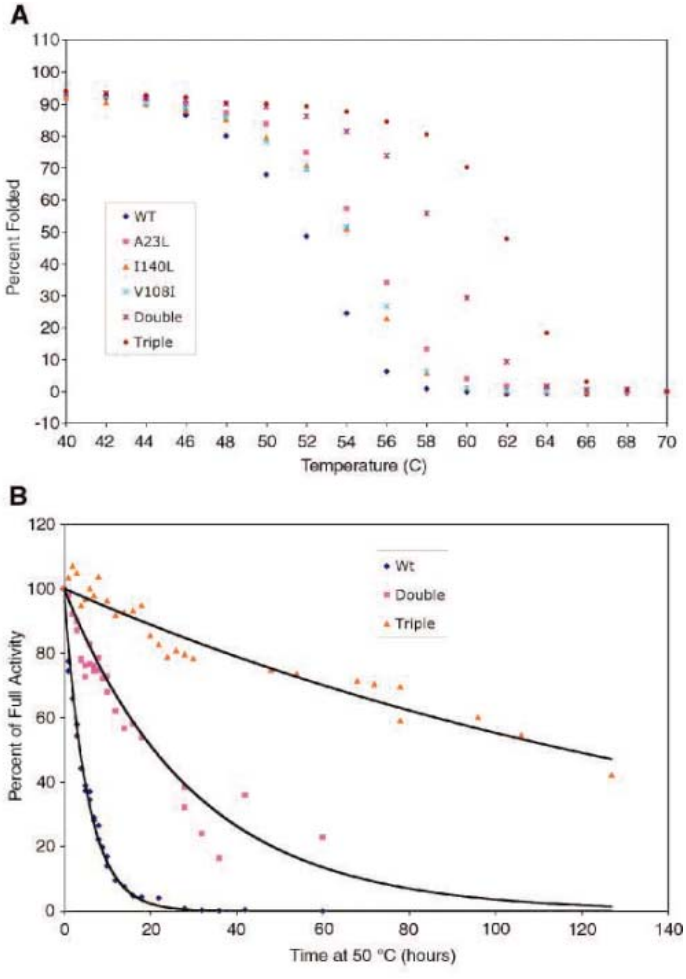

Fig. 1. Thermal denaturation and activity half-life measurements. (**A**) Temperature melt measuring the change in signal at 220 nm over a range of temperatures. All constructs show a folded baseline followed by a sigmodial two-state transition to an unfolded baseline. Only data from 40° to 70° are shown; at lower temperatures, the baseline plateaus corresponded to an assignment of 100% folded protein. (**B**) Activity decay at 50°C. Wild-type yCD and the double and triple mutant constructs were incubated at 50°C, and their activity was measured over time (*32*). The resulting curves gave half-lives for the enzymes at 50°C of 4 hours for the wild type (WT), 21 hours for the double mutant, and 117 hours for the triple mutant.

Table 1. Kinetic behavior of wild-type and redesigned yCD catalysts. Both the double and triple mutants displayed a slightly reduced V_{max}, coupled with a reduction in the Michaelis constant K_m. The catalytic efficiency of the various enzyme mutants (expressed as the ratio k_{cat}/K_m) was unchanged relative to the wild-type enzyme. M prod, molar concentration of product; enz, enzyme.

	Wild type	Double mutant	Triple mutant
K_m (mM)	1.98	1.50	1.33
V_{max} (M prod s^{-1})	0.00016	0.00012	0.00011
k_{cat} (M prod M enz^{-1} s^{-1})	160	120	110
k_{cat}/K_m (M enz^{-1} s^{-1})	80800	80000	82700

selecting mutations that differ in these properties, as compared to selection or redesign experiments based on natural selection.

In order to visualize the time-dependent decay of activity at elevated temperatures, wild-type yCD and the double and triple mutants were incubated at 50°C, and the decrease in their relative activity was monitored over time (Fig. 1B). The wild-type enzyme showed a rapid loss of activity at 50°C, with a half-life of ~4 hours. The double mutant displayed a half-life of ~21 hours, whereas the triple mutant had a half-life at 50°C of ~117 hours (a 30-fold increase over that of the wild type).

In order to determine the effects of the mutations in vivo, a strain of *Escherichia coli* dependent on cytosine deaminase function for uracil synthesis was engineered and transformed with both wild-type and mutant yCD reading frames. Doubling times were then measured at 30°C and 37°C on minimal media lacking uracil (Fig. 2). The thermostabilized mutant construct induced slightly accelerated growth relative to the wild-type enzyme at 30°C and a clear acceleration at 37°C. This suggests that the properties of the engineered variants (a reduced K_M and thermostabilization) measured in vitro correlate with improved enzyme flux in vivo under growth conditions limited by the activity of the enzyme.

The crystal structures of both the double and triple mutants were solved to 1.9 Å and 1.7 Å, respectively. The interpretation of density around the redesigned regions of the protein core (in unbiased omit maps) was unambiguous (fig. S4). The root mean square deviation values comparing the wild-type enzyme and both constructs were under 0.5 Å on all common α carbons and under 0.8 Å on all common atoms. Thus, the redesign and subsequent incorporation of point mutations in the enzyme core had a negligible effect on overall structure of the enzyme, including the active site (fig. S4). The redesigned, mutated residues all appear to pack more tightly in the enzyme core, with more surface area in contact with neighboring residues without altering the nearby side chain rotamers or backbone conformation. Approximately 70 Å^2 of additional buried surface area is incorporated as a result of the three mutations [based on an analysis of residue-by-residue packing, using the program NACCESS (*37*)]. The A23L/I140L double mutation increased the amount of hydrophobic packing against a neighboring tyrosine ring (Fig. 3A), and the addition of V108I in the triple mutant added an additional methyl group to fill a cavity (Fig. 3B).

The stabilized triple mutant was pieced together from part of a cluster of mutations predicted by the program and another single mutation predicted in a separate part of the core. Although the degree of thermostabilization produced by these mutations was relatively modest (an increase for T_m of 2°C for the first change and 4°C for each subsequent mutation), there is no obvious reason why additional mutations predicted by the program could not be iteratively incorporated into the enzyme core, resulting in a panel of catalysts that display sequential increases in thermal stability.

Not all mutations predicted by the program were equally thermostabilizing. Redesigns involving incorporation or alteration of polar or charged residues in the core (such as replacement of a buried salt-bridge in cluster 1 and individual mutations T67E, E69L, and W10T) were less successful than mutations involving substitution of one hydrophobic side chain for another. These latter mutations were predicted and observed to fill cavities within the core with additional van der Waals packing interactions. In future design efforts, selecting mutations of this type in silico may be most successful. Furthermore, modeling of interactions involving buried polar and charged side chains in the enzyme core is an area for future development in computational redesign algorithms.

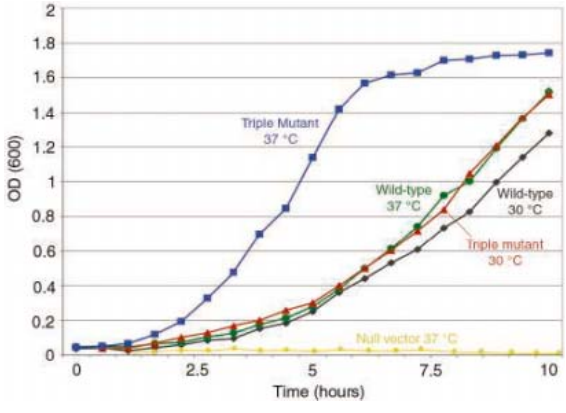

Fig. 2. In vivo assay for metabolic growth phenotype showing bacterial growth curves in media conditions requiring cytosine deaminase activity for generation of uracil (*32*). Both wild-type and reengineered mutants of yCD complement the bacterial activity; the thermostabilized enzyme variant displayed a slight increase in growth rate at 30°C and a clear increase at 37°C. OD (600), optical density at 600 nm.

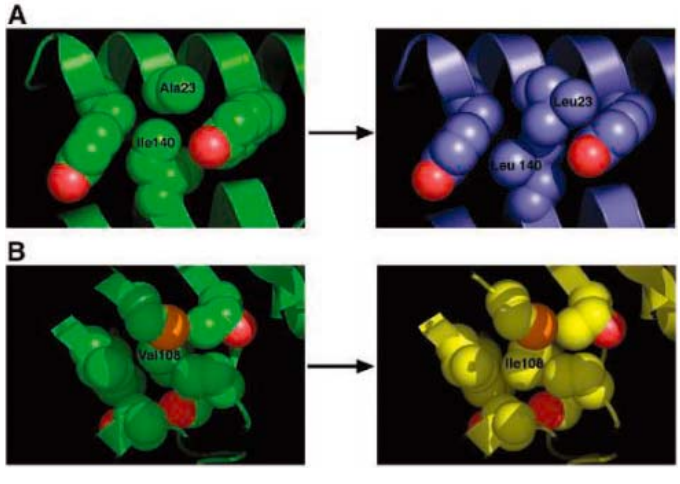

Fig. 3. Structural analyses. (**A**) Van der Waals representation of residues Y19, A23, Y26, and I140 in the wild-type yCD crystal structure (left) and the same representation and orientation for the mutant construct with A23L and I140L mutations (right). (**B**) Van der Waals radii representation of the area around V108 in the wild-type structure (left) and a similar representation of the area around the V108I mutation in the triple mutant crystal structure.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/308/5723/857/DC1
Materials and Methods
Figs. S1 to S4
Table S1
References and Notes

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Swimming Against the Flow: A Mechanism of Zooplankton Aggregation

Amatzia Genin,[1]* Jules S. Jaffe,[2] Ruth Reef,[1] Claudio Richter,[3] Peter J. S. Franks[2]

Zooplankton reside in a constantly flowing environment. However, information about their response to ambient flow has remained elusive, because of the difficulties of following the individual motions of these minute, nearly transparent animals in the ocean. Using a three-dimensional acoustic imaging system, we tracked >375,000 zooplankters at two coastal sites in the Red Sea. Resolution of their motion from that of the water showed that the animals effectively maintained their depth by swimming against upwelling and downwelling currents moving at rates of up to tens of body lengths per second, causing their accumulation at frontal zones. This mechanism explains how oceanic fronts become major feeding grounds for predators and targets for fishermen.

Buoyant phytoplankton and nonliving flotsam accumulate at the sea surface along convergent fronts because they remain afloat while the water submerges (*1*, *2*). Accumulations at fronts have also been reported for zooplankton (*3*, *4*); however, their aggregations often occur below the surface and at both convergent (downwelling) and divergent (upwelling) zones (*5*). Hardy (*6*) was the first to suggest that such patchiness must be caused by some dynamic principles involving zooplankton behavior and water movement. A common, yet untested explanation for subsurface accumulations at frontal zones is that the animals actively swim against vertical currents in an attempt to maintain their depth (*5*, *7*–*10*). Models (*7*, *8*) show that complete depth retention by zooplankton should generate increasingly dense accumulations, whereas partial retention, due to fatigue or inability to match the velocity of the current, should lead to ephemeral patches. Copepods can form fine-scale aggregations in layers where the turbulence velocity is substantially weaker than their typical swimming speed (*11*). Although diel vertical migrations are well known among zooplankton in response to seasonal cues, their behavioral response to ambient currents has not been demonstrated in the ocean, largely because of the lack of a technology that can track in situ the motions of these small, nearly transparent organisms in a large volume of water.

We tested the hypothesis that zooplankton swim against vertical currents by acoustically tracking animals while simultaneously measuring currents at two coastal sites in the northern Gulf of Aqaba, Red Sea (table S1). The sites experience persistent (hours-long) periods of upwelling and downwelling driven by differential heating and cooling across the gradually sloping bottom (*12*, *13*) and by the interaction between mesoscale currents and coastal topography. The three-dimensional trajectories of individual zooplankters as small as 1 mm in length were measured with FishTV-1.6 (FTV-1.6), a new, high-frequency (1.6-MHz), multibeam sonar (*14*), within volumes of water up to 3.8 m in length and 0.1 to 0.4 m in width (Fig. 1) (*15*). The sonar's transducer, attached to a large submerged tripod (Fig. 1), was varied in depth and orientation among deployments (table S1). Three experiments using FTV-1.6 accomplished 274 tracking sessions, most of them acquiring >10 min of uninterrupted bioacoustic data at a rate of three "frames" per second, yielding a total of 375,171 tracks. The sessions were performed day and night under conditions of upwelling and downwelling currents (table S1).

Vertical and horizontal currents at the depth of the insonified volume were measured nearby (<15 m) during each tracking session (Fig. 1) (*15*). The average vertical flow during the three experiments, approximately 1 cm/s, was 10 to 15% of the prevailing horizontal currents.

Net tows near the insonified volume indicated that during the day, zooplankton assemblages consisted mostly of pelagic species typical of the open waters of the Red Sea (*16*). At night, the emergence of demersal zooplankton doubled the zooplankton density (*17*). At all times, copepods were the dominant group (50 to 85% by number). Additional common taxa included mollusks, chaetognaths, and tunicates; and during the night, decapod larvae and other crustaceans. More than 70% of the targets recorded by FTV-1.6 had weak reflectivity (<–80 dB referenced to 1 μPa at 1 m range), in agreement with the dominance in the net samples of small (<5 mm) zooplankton from the aforementioned taxa.

Comparison of the tracks obtained from the sonar with the currents revealed that under both downwelling and upwelling conditions, the zooplankton swam against the vertical currents (Fig. 2 and Table 1). Complete depth retention, with a regression coefficient of –1.0 between the zooplankton's vertical swimming velocity (V_z, relative to water) and the vertical current (V_w), was found in the first and second experiments; and nearly complete retention ($V_z = -0.82\ V_w$) was found in the third experiment (Table 1). These results indicated that under strong vertical velocities, the small zooplankton recorded with FTV-1.6 swam vertically at velocities of >10 body lengths/s. In contrast, the animals' mean horizontal displacement (H_z) was indistinguishable from that of the current (H_w), with a regression coefficient of 1.0 ($H_z = H_w$) in the first and second experiments, indicating that the animals were passively drifting with horizontal currents; and nearly so ($H_z = 0.73\ H_w$) in the third experiment (Fig. 2).

Planktonic organisms that maintain their depth are expected to accumulate where vertical currents persist (*7*, *8*). Because shallow downwelling and upwelling zones at the study sites were confined to near-shore waters (*12*, *13*), a greater abundance of zooplankton was expected near the coast (movie S1). To test this prediction, we examined the distri-

[1]The Interuniversity Institute for Marine Sciences and the Hebrew University, Eilat, Israel. [2]Scripps Institution of Oceanography, La Jolla, CA, USA. [3]Center for Tropical Marine Ecology, Bremen, Germany.

*To whom correspondence should be addressed. E-mail: amatzia@vms.huji.ac.il

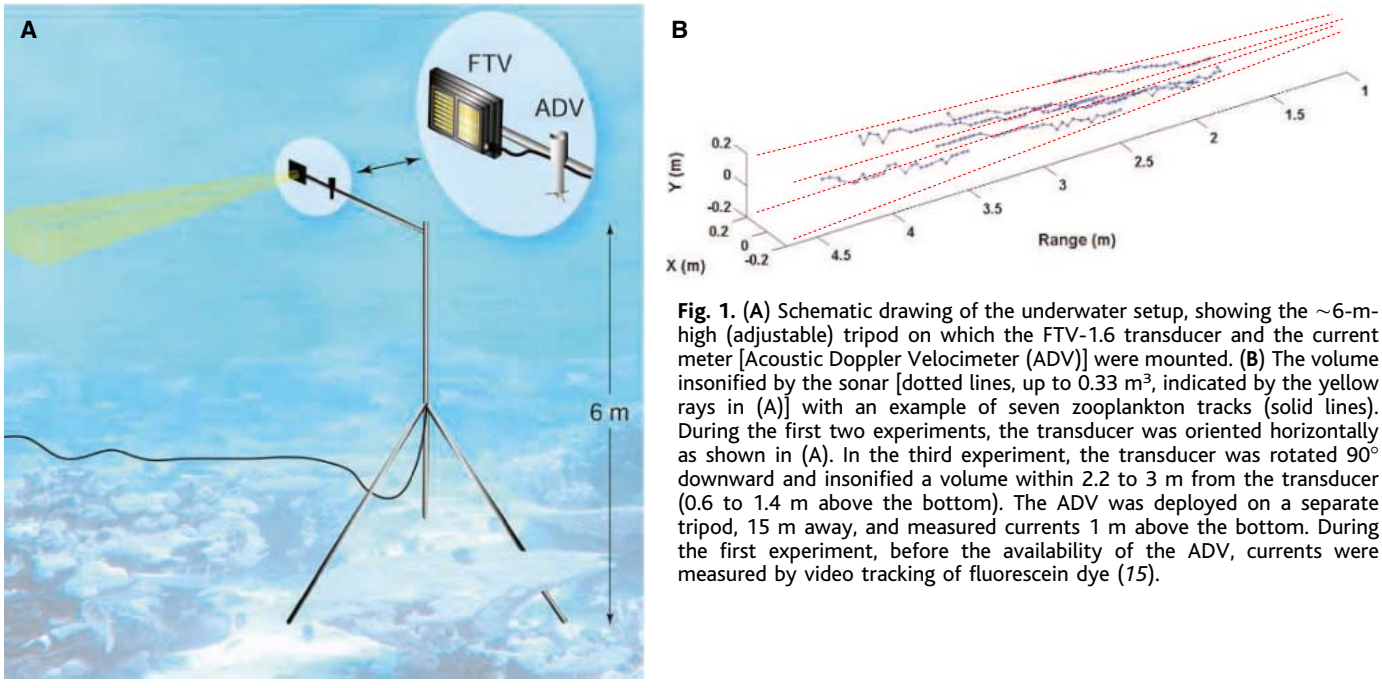

Fig. 1. (**A**) Schematic drawing of the underwater setup, showing the ~6-m-high (adjustable) tripod on which the FTV-1.6 transducer and the current meter [Acoustic Doppler Velocimeter (ADV)] were mounted. (**B**) The volume insonified by the sonar [dotted lines, up to 0.33 m^3, indicated by the yellow rays in (A)] with an example of seven zooplankton tracks (solid lines). During the first two experiments, the transducer was oriented horizontally as shown in (A). In the third experiment, the transducer was rotated 90° downward and insonified a volume within 2.2 to 3 m from the transducer (0.6 to 1.4 m above the bottom). The ADV was deployed on a separate tripod, 15 m away, and measured currents 1 m above the bottom. During the first experiment, before the availability of the ADV, currents were measured by video tracking of fluorescein dye (*15*).

bution of zooplankton across the sandy shore of Ras Burka (experiment 1, table S1). The reef site could not be used for this test because of intense predation on zooplankton by reef fishes and invertebrates (*18*). Three sets of zooplankton sampling were conducted, each consisting of four net tows at different distances from shore (*15*). Two sets were done during periods of downwelling, and one was done when there was no discernible vertical current. Zooplankton density was approximately three times greater near the downwelling front than in the offshore waters (Fig. 3); no obvious cross-shore pattern was observed when vertical currents were negligible. The cross-shore pattern during downwelling agreed with that predicted by a simulation model (*7*, *8*) using local bathymetry and observed downwelling velocities as input parameters. The simulation indicated that it would take 0.5 to 2.7 hours for depth-keeping animals to triple their density near the shore in the presence of downwelling of 3.6 and 0.4 cm/s, respectively (Fig. 3). Downwelling typically persists for several hours at our study sites (*12*, *13*).

The mechanism used by the zooplankton to sense their depth is currently unknown. Because depth retention was observed at all times, including moonless nights, light is unlikely to be the universal cue eliciting this behavior, although at the shallow depths at which we worked, crustaceans can sense light intensity even at night (*19*). One possibility is that the animals are sensing pressure. Although pressure sensors have not been identified in copepods, frontal organs of unknown function are found in these organisms (*20*), and some copepods as well as other crustaceans respond behaviorally to small changes in pressure (*21*–*24*). Those findings were interpreted as indicating a "biological barostat" by which the animals might maintain constant depth (*25*). The fact that a cellular-level mechanism for pressure sensing is found in many invertebrates (*26*) makes pressure an intriguing possibility as a cue for directional swimming and depth maintenance in zooplankton.

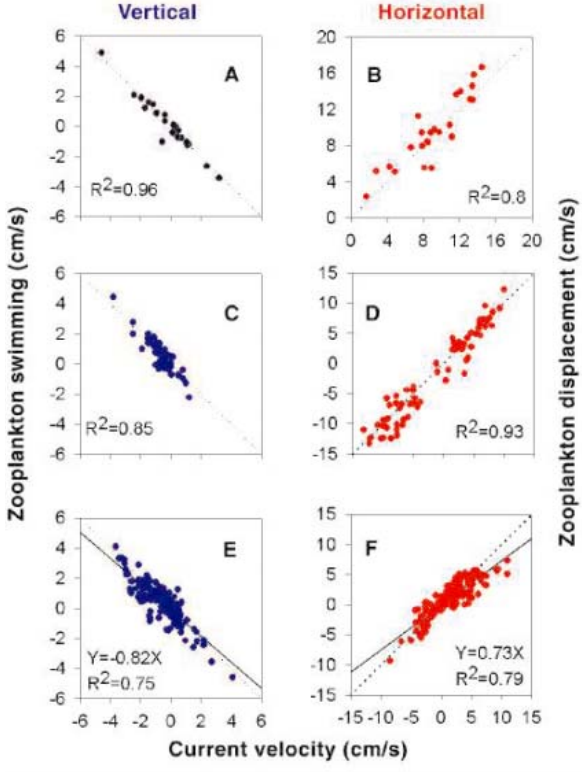

Fig. 2. Zooplankton motion versus water velocity along vertical (left panels) and horizontal (right panels) directions during the first (**A** and **B**), second (**C** and **D**), and third (**E** and **F**) experiments. Zooplankton vertical motions are presented as swimming velocities (relative to water), and horizontal motions are absolute (relative to Earth). Each data point indicates the average value of a tracking session (5 to 14 min, 200 to >20,000 measurements). The 95% confidence intervals around each point (not shown) are typically much smaller than the size of the symbol. Dotted lines indicate negative ($y = -x$, left panels) and positive ($y = x$, right panels) linear relationships between the parameters. The regression lines between the plotted variables, for which the R^2 values are shown, were highly significant ($P < 0.001$) in all six panels. Solid lines in (E) and (F) indicate the regression lines in the two cases where their slope was different from that of the dotted line. The video dye method of measuring currents during the first experiment (B) allowed the calculation of horizontal speed (scalar), not velocity (*15*).

The adaptive benefits of depth retention are not well understood. Ultimately, such behavior is necessary to avoid a passive drift into unfavorable depths. Depth preference during ontogenetic development is an effective mechanism for directed horizontal transport in some types of flow (*27*). Depth-keeping could also be an effective strategy to remain

within thin layers of high food concentrations (*28*), especially if such layers disperse when advected vertically. In addition, because depth-keeping in vertical flows leads to patch formation (*7*, Fig. 3), this behavior enhances the probability of finding a mate in an otherwise sparsely populated ocean.

The formation of zooplankton patches at fronts has far-reaching implications for their predators. The survival and growth of many zooplankton predators, from invertebrates to whales, depends on their success in finding rich patches of prey (*29–32*); the ambient abundances of zooplankton outside these patches are often too low to maintain the observed rates of predator growth and reproduction (*31*).

If depth retention is pervasive, it could be a key mechanism for the formation of zooplankton patches that predators can dependably locate by tracking well-defined cues (such as a sharp temperature gradient across a front) or foraging in regions of persistent fronts (*33*). The numerous observations of dense aggregations of zooplankton and their predators at sites of vertical currents (*5*), including mid-ocean fronts (*3*), shelf breaks (*9*), submarine canyons (*33*), and banks (*34*), indicate that the phenomenon is widespread. Fishermen also frequent fronts (*35*). Hence, the zooplankton's ability to swim against the flow appears to have a major effect on the ability of pelagic predators to thrive in an otherwise food-impoverished ocean.

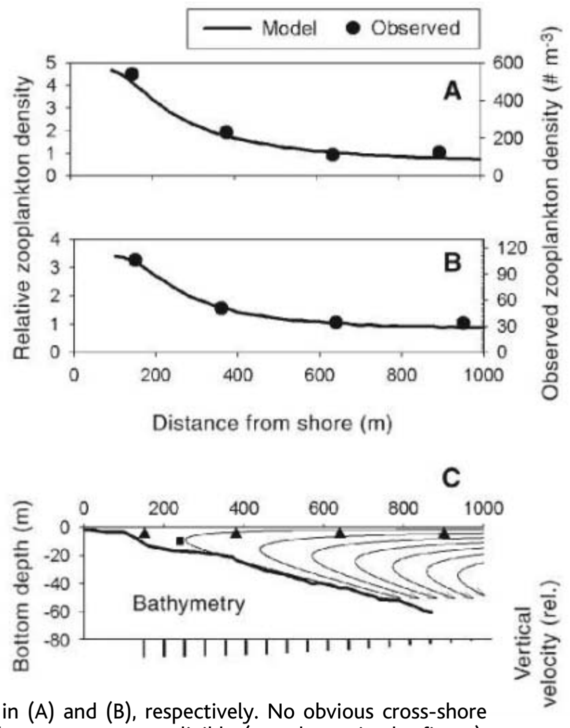

Fig. 3. Observed (circles) and predicted (lines) zooplankton densities at 3 to 4 m depth across the shore at Ras Burka during downwelling of 0.4 cm/s (**A**) and 3.6 cm/s (**B**) on 5 and 6 December 1998, respectively. (**C**) The corresponding cross-shore profile of bottom depth (thick line), the stream lines generated by equations 2 and 3 in (*7*) (thin lines), and the vertical velocity at different distances from shore (vertical bars under the bottom axis). The stream lines are plotted evenly spaced on the stream function, so that more condensed stream lines indicate faster flow. Simulated density and vertical velocity are in arbitrary units relative to those farthest from shore. The simulation indicates that under the downwelling velocities measured at our deployment site [square in (C)] during the zooplankton net tows [tow positions are indicated by triangles in (C)], it would take 2.7 and 0.5 hours for depth-keeping animals to generate the cross-shore patterns plotted in (A) and (B), respectively. No obvious cross-shore pattern was observed when vertical currents were negligible (not shown in the figure). See movie S1 for an animated simulation of the accumulation mechanism.

Table 1. Results of the three zooplankton-tracking experiments. Mean (±SD) vertical velocities of currents, zooplankton displacement (relative to Earth), and results of the regression analysis between the zooplankton's vertical swimming velocity (V_z, relative to water) and the vertical current velocity (V_w) under conditions of upwelling, downwelling, and both in each of the three experiments are shown. *n*, number of tracking sessions. *P* value, significance level of the regression coefficient (*A*) as follows: $**P < 0.001$, $***P < 0.00001$. SE, standard error of the regression coefficient. In 3 of the 65 sessions of the second experiment, the vertical current was nearly zero.

Experiment	Water current (cm/s)	Zooplankton displacement (cm/s)	*n*	Regression [$V_z = AV_w$]		
				A (SE)	R^2	*P* value
Upwelling						
1	1.37 (±1.7)	–0.05 (±0.2)	13	–1.0 (0.03)	0.99	***
2	0.56 (±0.42)	–0.11 (±0.54)	9	–1.33 (0.24)	0.79	**
3	0.69 (±0.77)	0.01 (±0.55)	56	–1.11 (0.07)	0.82	***
Downwelling						
1	–1.5 (±1.2)	–0.14 (±0.54)	11	–0.98 (0.09)	0.92	***
2	–0.89 (±0.66)	–0.05 (±0.4)	53	–1.0 (0.05)	0.87	***
3	–1.16 (±0.87)	–0.3 (±0.67)	129	–0.75 (0.04)	0.75	***

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Supporting Online Material

www.sciencemag.org/cgi/content/full/308/5723/860/DC1
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Target Cell–Dependent Normalization of Transmitter Release at Neocortical Synapses

Helmut J. Koester[1,2*†] and Daniel Johnston[2*]

The efficacy and short-term modification of neocortical synaptic connections vary with the type of target neuron. We investigated presynaptic Ca^{2+} and release probability at single synaptic contacts between pairs of neurons in layer 2/3 of the rat neocortex. The amplitude of Ca^{2+} signals in boutons of pyramids contacting bitufted or multipolar interneurons or other pyramids was dependent on the target cell type. Optical quantal analysis at single synaptic contacts suggested that release probabilities are also target cell–specific. Both the Ca^{2+} signal and the release probability of different boutons of a pyramid contacting the same target cell varied little. We propose that the mechanisms that regulate the functional properties of boutons of a pyramid normalize the presynaptic Ca^{2+} influx and release probability for all those boutons that innervate the same target cell.

Synapses are believed to be important sites for learning and memory. Synapses between neurons are heterogeneous in synaptic efficacy and show distinct forms of short-term plasticity. One determinant of synaptic efficacy and short-term plasticity is the probability of neurotransmitter release. The rate and type of information transfer across a synapse are dependent on release probability and short-term plasticity (*1*, *2*). Furthermore, the computational function of neurons within a network depends on the form of short-term plasticity of the synapses they receive (*3*, *4*). Release probability has not been directly determined in synaptic connections in situ, only for autapses in tissue culture (*5*). The small size of synapses, their electrotonic isolation from somata, and the high density of cellular structures in brain tissue has prevented the direct measurement of release probability among synapses of connected pairs of neocortical neurons.

Synaptic connections between pyramids and interneurons in layer 2/3 (L2/3) of the somatosensory cortex of young rats have different efficacy, reliability, and short-term plasticity (*6*–*8*). These differences may depend on presynaptic factors such as influx of calcium ions (Ca^{2+}), Ca^{2+} buffering and diffusion in the cytoplasm, the Ca^{2+} sensor for release, and Ca^{2+} extrusion (*8*–*10*). Consistent with this view, the boutons of pyramid axons in L2/3 show a relatively wide range of action potential (AP)–evoked Ca^{2+} signal amplitudes, varying up to 10-fold between different boutons of the same axonal arbor (*11*–*13*). A synaptic connection between neocortical cells is, in most cases, formed by several synaptic contacts (*14*–*17*), and their individual properties are unknown.

To elucidate how a single contact contributes to the net behavior of a synaptic connection between two neurons and to examine presynaptic determinants of target-cell specificity, we used high-resolution fluorescence imaging (*18*–*20*) to examine pre- or postsynaptic AP-evoked Ca^{2+} transients in 63 single synaptic contacts. Synaptically connected pairs of neurons in L2/3 of the somatosensory neocortex of young rats were examined in acute brain slices by dual whole-cell voltage recordings under visual control (*21*, *22*). Pre- and postsynaptic cells of a pair were classified based on discharge pattern and dendritic morphology (*6*, *7*). To examine Ca^{2+} signals of pyramidal cell boutons contacting different target cells, we loaded presynaptic pyramids with Oregon Green 488 Bapta-1 (OGB-1, 200 μM) and postsynaptic neurons with Alexa 594 (Fig. 1A). Contacts were identified by (i) the overlap of an axon of the presynaptic pyramid and a dendrite of the postsynaptic cell, and (ii) the presence of an axonal varicosity where axons and dendrites were apposed (Fig. 1B). The fact that, in most cases, appositions selected in this way were indeed functional synaptic contacts was verified by postsynaptic Ca^{2+} imaging (see below).

Pyramid-to-bitufted (P-B) connections (Fig. 1) formed mostly axospinous contacts (>60%). We examined those bitufted interneurons that showed frequency adaptation of APs and facilitation of excitatory postsynaptic potentials (EPSPs) in response to evoked APs in the presynaptic pyramid. These connections had low efficacy and high failure rates and showed paired-pulse facilitation (Fig. 1C and Table 1).

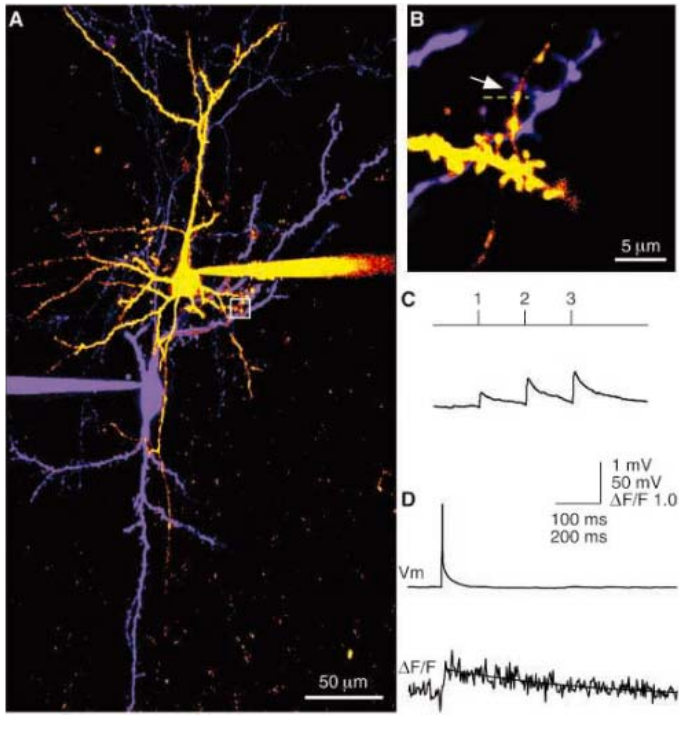

Fig. 1. Identification of single synaptic contacts in P-B connections. (**A**) Overlay of an L2/3 pyramidal neuron filled with OGB-1 (yellow, pseudocolor) and a bitufted interneuron filled with Alexa 594 (blue, pseudocolor). Recording pipettes are attached to somata. The white square indicates the location of the synaptic contact in (B). (**B**) A synaptic contact (arrow) examined for presynaptic Ca^{2+} signals in response to presynaptic APs. The broken yellow line indicates the position of the line scan. (**C**) A train of APs (three APs at 10 Hz), evoked by brief current injections in the presynaptic pyramidal cell, elicited EPSPs in the postsynaptic interneuron (lower trace, average of 20 sweeps) that successively increased in amplitude. (**D**) An AP in the presynaptic pyramid (upper trace) evoked a small Ca^{2+} fluorescence signal (lower trace) in the bouton at the synaptic contact illustrated in (B). The continuous line represents a single exponential fit. The Ca^{2+} signals shown do not reflect the physiological amplitude and time course of $[Ca^{2+}]_i$ in a bouton, because the indicator OGB-1 acts as an exogenous Ca^{2+} buffer (*28*, *29*).

[1]Abteilung Zellphysiologie, Max-Planck-Institut für Medizinische Forschung, Jahnstraße 29, D-69120 Heidelberg, Germany. [2]Department of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.

*Present address: Center for Learning and Memory, University of Texas at Austin, Austin, TX 78712, USA. †To whom correspondence should be addressed. E-mail: HKoester@mail.utexas.edu

Ca^{2+} signals in boutons of contacts of these connections, evoked by a single presynaptic AP, were small [relative calcium fluorescence change $\Delta F/F = 0.54 \pm 0.29$, mean ± SD (*22*), $n = 12$ boutons] (Fig. 1D) compared to the average AP-evoked Ca^{2+} signal in pyramidal cell boutons measured under the same conditions [$\Delta F/F = 1.31 \pm 0.68$ (*12*)]. Pyramid-to-multipolar (P-M) connections had different characteristics (Fig. 2). Contacts were mostly axodendritic (>80%) and located close to the (postsynaptic) soma; the failure rate was low; synaptic efficacy was high; and evoked EPSPs showed paired-pulse depression (Fig. 2C and Table 1). AP-evoked presynaptic Ca^{2+} signals had an average amplitude of $\Delta F/F = 1.62 \pm 0.43$ ($n = 11$ boutons), about threefold as large as that measured in boutons of P-B connections. This difference was significant [$P < 0.01$, analysis of variance (ANOVA)]. Finally, pyramid-to-pyramid (P-P) connections also had low failure rates (Table 1). P-P connections usually showed depression and only in a few cases (15%) displayed paired-pulse facilitation. P-P contacts were mostly axospinous (>60%). Presynaptic $[Ca^{2+}]_i$ (intracellular calcium concentration) transients ranged from $\Delta F/F = 0.25$ to 2.28; the average amplitude was $\Delta F/F = 0.96 \pm 0.67$ ($n = 8$ boutons, $P < 0.05$ compared to P-M connections, ANOVA). We did not find significant differences in the presynaptic Ca^{2+} signal between axospinous and axodendritic contacts for any of the three classes of connections between pyramids and different target cell types ($P > 0.05$, t tests). The low z-resolution did not allow us to identify spines reliably in all cases, and thus the number of axospinous contacts is underestimated. Connections with larger presynaptic Ca^{2+} signals had a lower EPSP failure rate (Fig. 2E).

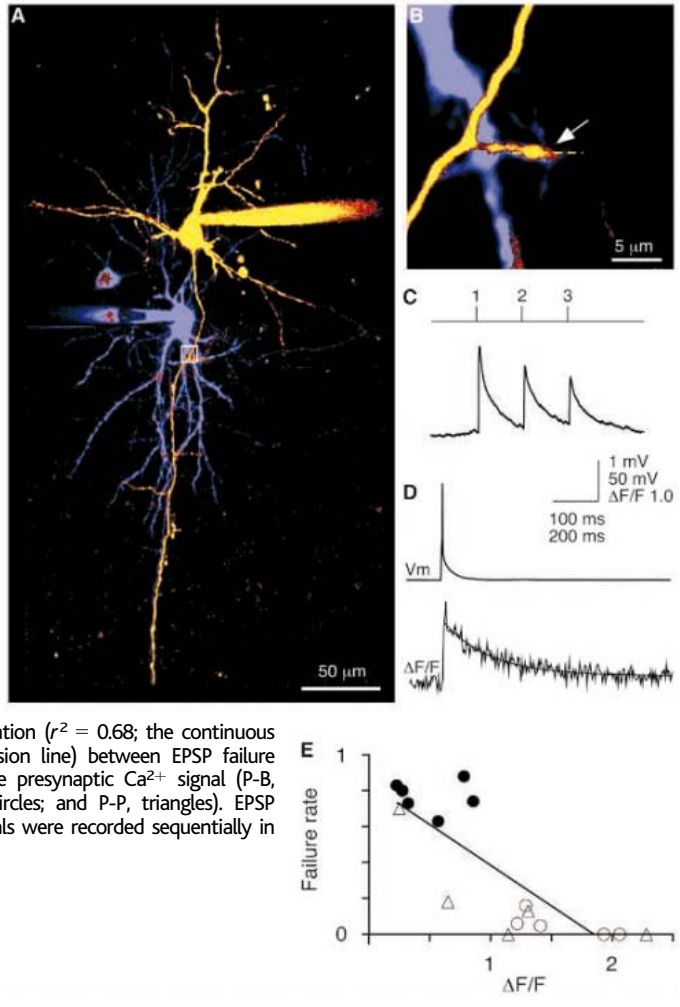

Fig. 2. Identification of single synaptic contacts in P-M connections. (**A**) Overlay of an L2/3 pyramidal neuron filled with OGB-1 (yellow, pseudocolor) and a multipolar interneuron filled with Alexa 594 (blue, pseudocolor). (**B**) A synaptic contact examined for a presynaptic Ca^{2+} signal in response to a presynaptic AP. The broken yellow line indicates the position of the line scan. (**C**) A presynaptic train of APs (three APs at 10 Hz) elicited EPSPs in the postsynaptic multipolar interneuron (lower trace, average of 20 sweeps) that decreased in amplitude. (**D**) An AP in the presynaptic pyramid (upper trace) evoked a large Ca^{2+} fluorescence signal in the bouton at the synaptic contact illustrated in (B). (**E**) Graph of the linear correlation ($r^2 = 0.68$; the continuous line represents the regression line) between EPSP failure rate and amplitude of the presynaptic Ca^{2+} signal (P-B, solid circles; P-M, open circles; and P-P, triangles). EPSP failure rate and Ca^{2+} signals were recorded sequentially in each pair.

The three classes of connections not only differ in reliability but also in frequency-dependent short-term plasticity. The presynaptic Ca^{2+} fluorescence transients evoked by a single AP and by trains of three APs (at 10 Hz) in P-B connections had similar amplitudes. The amplitude ratios of the second and third Ca^{2+} transient to the first were, on average, 96 ± 42% and 103 ± 24% [$n = 7$ boutons], respectively. In contrast, in P-M connections, the second and third APs evoked a fluorescence signal with peak amplitudes of 94 ± 17% and 83 ± 23% ($n = 9$ boutons), compared to the first AP. In P-P connections, the second and third amplitudes were 97 ± 28% and 84 ± 24% ($n = 4$ boutons). This decrease in amplitude for P-M and P-P connections, however, is presumably due to saturation of the high-affinity Ca^{2+} indicator OGB-1. This was indicated by the inverse correlation of the relative amplitude of the third response with the amplitude of the first ($r^2 = 0.48$ and $r^2 = 0.61$). Furthermore, in experiments with a low-affinity indicator in single-cell recordings, summation was always linear (*12*).

When we examined two or more synaptic contacts of the same connection, the variability in the amplitude of the Ca^{2+} signals between boutons was very small. The high correlation ($r^2 = 0.78$) of amplitudes measured in different boutons of the same connection indicated that the variability between boutons of a pair was small (Fig. 4C). Assuming that the volume-averaged Ca^{2+} signals were proportional to the initial fast Ca^{2+} influx that briefly increases the transmitter release probability (*23*), the small difference between different boutons of a particular connection suggested that the increase in release probability, evoked by a single AP, could be comparable for all release sites.

We tested this hypothesis in a second series of experiments by optical quantal analysis. Postsynaptic cells were filled with the Ca^{2+} indicator (OGB-1, 100 μM), and Alexa 594 was loaded into the presynaptic pyramid (Fig. 3). Potential synaptic contacts (Fig. 3B) were

Table 1. Properties of L2/3 synaptic connections. The table shows average values and the number of recordings (n) for connections between pyramids and various target cell types. The paired-pulse ratio was measured at 10 Hz from the ratio of the first and second peaks in a train of three APs. Pre- and postsynaptic distance refer to the geometric distance in xy (neglecting z) of a synaptic contact from pre- and postsynaptic soma.

Synapse property	Target cell type		
	L2/3 pyramid (n)	Multipolar (n)	Bitufted (n)
Unitary EPSP (mV)	0.92 ± 1.01 (34)	2.35 ± 1.40 (8)	0.22 ± 0.19 (22)
Failure rate (%)	22 ± 21 (34)	4 ± 6 (8)	69 ± 19 (22)
Paired-pulse ratio (%)	77 ± 35 (26)	66 ± 9 (8)	223 ± 126 (18)
Postsynaptic distance (μm)	55 ± 30 (21)	38 ± 37 (14)	89 ± 78 (23)
Presynaptic $\Delta F/F$	0.96 ± 0.67 (8)	1.62 ± 0.43 (11)	0.54 ± 0.29 (12)
Release probability/contact	0.46 ± 0.26 (17)	0.64 ± 0.16 (3)	0.13 ± 0.08 (12)

examined for a postsynaptic Ca^{2+} signal in response to a presynaptic AP (Fig. 3D). These fluorescence signals were a result of postsynaptic Ca^{2+} influx, presumably mediated by *N*-methyl-D-aspartate receptors (*20*, *24*). The Ca^{2+} signals were highly localized, and their occurrence varied stochastically, indicating successes and failures of presynaptic APs to elicit transmitter release (Fig. 3E). The optically measured release probability, p_r, ranged from 0.05 to 0.92 and was dependent on target cell type. Average postsynaptic resting membrane voltages in these experiments were –63 mV (P-B), –66 mV (P-M), and –67 mV (P-P). In P-B connections ($p_r = 0.13 \pm 0.08$, $n = 12$ contacts), release probability was significantly lower than in P-M connections ($p_r = 0.64 \pm 0.16$, $n = 3$ contacts, $P < 0.01$, ANOVA) or in P-P connections ($p_r = 0.46 \pm 0.26$, $n = 17$ contacts, $P < 0.01$, ANOVA). These differences suggest that, in addition to the target-cell specificity of Ca^{2+} transients, the release probability of individual pyramidal cell boutons was also target cell–dependent (Fig. 4). In agreement with the presynaptic Ca^{2+} signal measurements, we found no significant difference in release probability for axospinous and axodendritic contacts in the three classes of connections ($P > 0.05$, *t* tests).

When two synaptic contacts of the same connection were examined, their release probabilities were similar (Fig. 4D) ($n = 15$ pairs of contacts). The individual contacts were usually located on different branches of the dendritic and axonal arbors and thus were presumably independent. In no pair of contacts did we find a significant difference in release probability [tested with χ^2-statistics ($P < 0.05$) or by Fisher's exact probability test (for small release probabilities)]. We refer to this as normalization of release probability for different boutons of a connection. It was independent of the distance of contacts to each other (Fig. 4E). The release probabilities of two contacts of a connection were correlated for P-B connections ($r^2 = 0.77$) and for P-P connections ($r^2 = 0.91$).

Simulations of binomial and intersite variation showed that the distributions of release probabilities are consistent with a coefficient of variation of 0.16 for P-B and 0.19 for P-P connections (*22*). The experimental and simulation results indicate a small width for the distribution of release probabilities for all contacts within a given connection. These results suggest a model for neocortical synaptic connections that assumes binomial release, similar release probabilities, and varying quantal contents for the release sites. We tested a simple version of this model (the normalization model, assuming zero intersite variability in p_r) to see if it is consistent with the observed EPSP amplitude distributions. Using standard hypothesis testing (*25*, *26*), we could reject simple and compound binomial models underlying EPSP amplitude distributions in ~50% of the experiments, in favor of the normalization model (*22*).

The results document a target cell–specific difference of volume-averaged Ca^{2+} signals evoked by an AP in single boutons of L2/3 pyramidal cells. The size of the Ca^{2+} signal is substantially larger in connections with higher efficacy and reliability. A comparable target-cell specificity was found for optically measured release probabilities at individual synaptic contacts. Thus, both Ca^{2+} inflow and release probability of a bouton are target cell–specific. Target-cell specificity might be even more stringent than reported here, because interneuron classes, as defined by the AP discharge pattern and dendritic/axonal morphology, may represent an inhomogeneous cell population (*27*). Furthermore, the results are biased for those contacts that are located close (on average within <100 μm) to somata of both pre- and postsynaptic neurons, as a result of our searching procedure for contacts along the axon. Whether the difference between Ca^{2+} signals in boutons innervating different classes of target cells is a result of differences in Ca^{2+} channel density or channel subtypes, the endogenous Ca^{2+} buffer ratio, the size of these boutons, or a combination of these factors cannot be determined at present.

Different boutons of an individual connection had Ca^{2+} transients of similar amplitude, even if the boutons were located as far as 300 μm apart. This normalization of synaptic contacts of a connection was unexpected, because there is no obvious postsynaptic signal known at present that would operate over such large distances. Different boutons of a pyramid have a wide range of Ca^{2+} amplitudes even for nearby boutons of the same axon branch (*11*, *12*). In accordance with the idea of a normalization of presynaptic Ca^{2+} transients, we also found a normalization of release probabilities for different contacts of a connection. This suggests that one factor contributing to the normalization of release in all boutons of a layer 2/3 pyramidal cell that contact the same target cell is the size of the presynaptic Ca^{2+} influx. The normalization might be simply a result of target-cell specificity. However, there is no indication of such a fine subclassification of neurons.

We are therefore left with the conclusion that the normalization could arise from the unique pattern of pre- and postsynaptic activity that is similar for all contacts of a given

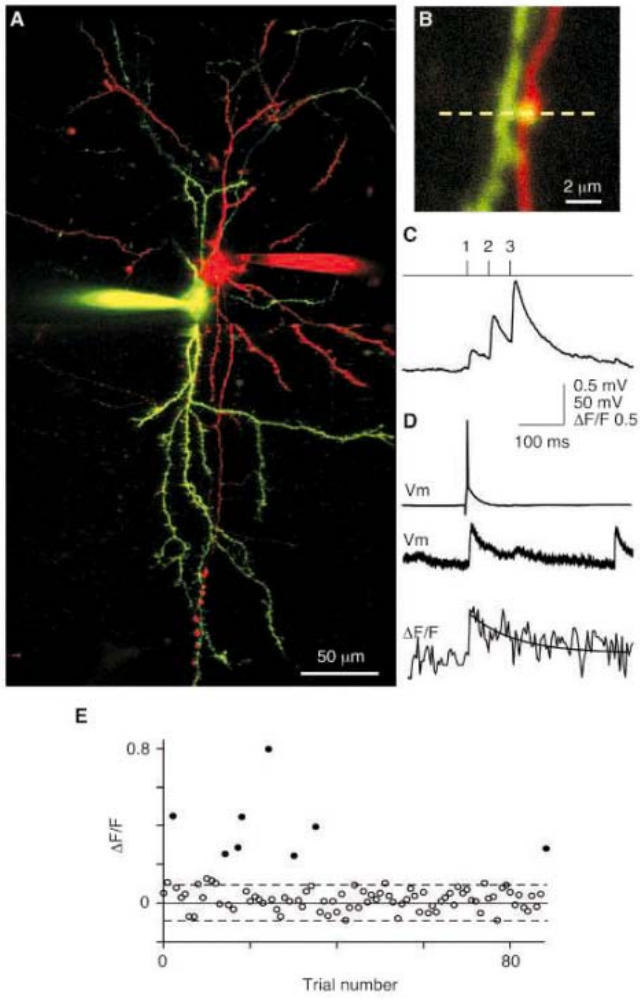

Fig. 3. Optical quantal analysis in a P-B cell connection. (**A**) Overlay of an L2/3 presynaptic pyramid filled with Alexa 594 (red, pseudocolor) and a postsynaptic bitufted neuron filled with OGB-1 (green, pseudocolor). The resting membrane voltage of the bitufted neuron was –59 mV. (**B**) Fluorescence image of a synaptic contact. The broken yellow line indicates the scan line. (**C**) Presynaptic stimulation of APs at 20 Hz (indicated by numbers) evoked facilitating EPSPs in the bitufted cell (average of 50 sweeps). (**D**) A single presynaptic AP (upper trace) evoked an EPSP in the postsynaptic cell (middle trace) and a Ca^{2+} fluorescence transient (lower trace) in the spine shown in (B). The continuous line represents a single-exponential fit to the data. (**E**) The graph shows the amplitude of the Ca^{2+} fluorescence signal ($\Delta F/F$) of all recordings in the spine shown in (B). Successes of transmission (solid circles) were easily distinguished from failures (open circles). Broken lines approximate the noise of the recordings.

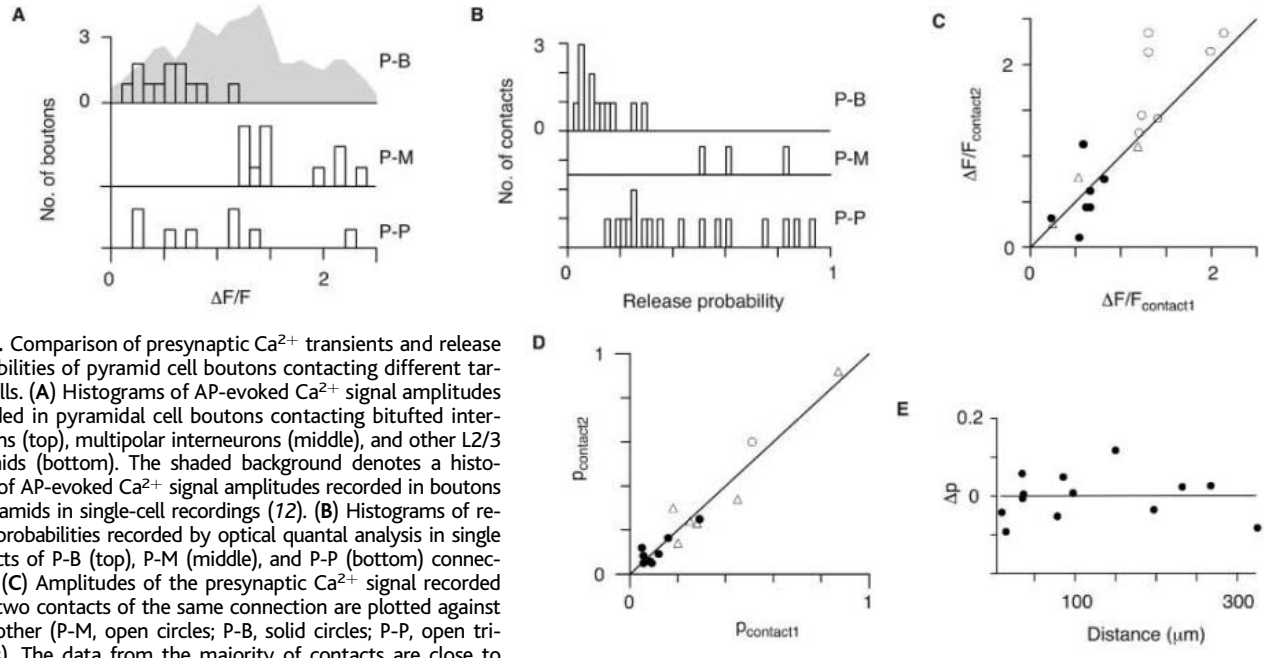

Fig. 4. Comparison of presynaptic Ca^{2+} transients and release probabilities of pyramid cell boutons contacting different target cells. (**A**) Histograms of AP-evoked Ca^{2+} signal amplitudes recorded in pyramidal cell boutons contacting bitufted interneurons (top), multipolar interneurons (middle), and other L2/3 pyramids (bottom). The shaded background denotes a histogram of AP-evoked Ca^{2+} signal amplitudes recorded in boutons of pyramids in single-cell recordings (*12*). (**B**) Histograms of release probabilities recorded by optical quantal analysis in single contacts of P-B (top), P-M (middle), and P-P (bottom) connections. (**C**) Amplitudes of the presynaptic Ca^{2+} signal recorded from two contacts of the same connection are plotted against each other (P-M, open circles; P-B, solid circles; P-P, open triangles). The data from the majority of contacts are close to the unity line (angular line). The correlation coefficient is $r^2 = 0.78$. (**D**) Release probability p_r recorded in one contact plotted against another contact of the same connection (P-M, open circles; P-B, solid circles; P-P, open triangles). All data points are close to the unity line (angular line). The correlation coefficient is $r^2 = 0.93$. (**E**) Graph showing the difference in optically measured release probability of two contacts of a connection plotted against the geometric distance of the two contacts. There is no correlation between these two parameters ($r^2 = 3 \times 10^{-5}$). The continuous line represents the regression line.

connection. Finally, the fact that P-P connections have the largest variability in their release probability may suggest that they can be more readily changed in the long term, for example as a function of usage.

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Nicotinic Acid Limitation Regulates Silencing of *Candida* Adhesins During UTI

Renee Domergue,[1] Irene Castaño,[1]* Alejandro De Las Peñas,[1]* Margaret Zupancic,[1] Virginia Lockatell,[2] J. Richard Hebel,[3] David Johnson,[2,4] Brendan P. Cormack[1]†

The adherence of *Candida glabrata* to host cells is mediated, at least in part, by the *EPA* genes, a family of adhesins encoded at subtelomeric loci, where they are subject to transcriptional silencing. We show that normally silent *EPA* genes are expressed during murine urinary tract infection (UTI) and that the inducing signal is the limitation of nicotinic acid (NA), a precursor of nicotinamide adenine dinucleotide (NAD^+). *C. glabrata* is an NA auxotroph, and NA-induced *EPA* expression is likely the result of a reduction in NAD^+ availability for the NAD^+-dependent histone deacetylase Sir2p. The adaptation of *C. glabrata* to the host, therefore, involves a loss of metabolic capacity and exploitation of the resulting auxotrophy to signal a particular host environment.

In the United States, *Candida albicans* and *C. glabrata* are the primary and secondary causes of both bloodstream and mucosal candidiasis (*1*, *2*). *Candida* accounts for about 25% of all urinary tract infections (UTIs) related to indwelling catheters, with *C.*

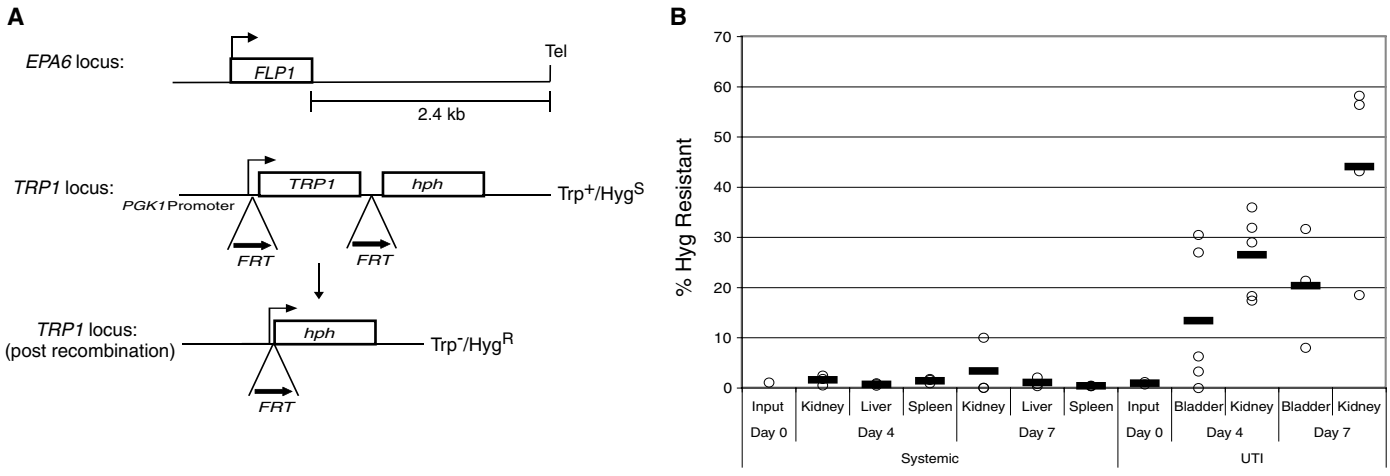


Fig. 1. *FLP* recombinase reporter. (**A**) Schematics of the *FLP1* replacement at the *EPA6* locus and the *PGK1/FRT/TRP1/FRT/hph* reporter, showing pre- and post-recombination configurations. (**B**) Hyg^R phenotype (expressed as a percentage of total cells recovered) of *C. glabrata* strain BG1087 recovered from organs after bloodstream infection or UTI. Each symbol (circles) corresponds to the *C. glabrata* cells recovered from one animal. Bars represent the mean.

glabrata accounting for approximately 15% of all *Candida* isolates (*3*). Adherence to host cells is likely important in the virulence of *Candida* species (*4*). In *C. glabrata*, adherence to epithelial cells in vitro is mediated by a lectin that is encoded by the *EPA1* gene (*5*). *EPA1* is part of a gene family in *C. glabrata*, most members of which are encoded in subtelomeric loci, where they are subject to *SIR*-dependent transcriptional silencing (*6*). In *Saccharomyces cerevisiae*, silencing at telomeres occurs by recruitment of the Sir complex (Sir2p, Sir3p, and Sir4p) to the telomeric repeats, followed by spread into adjacent subtelomeric regions (*7*). In *C. glabrata sir3*Δ mutants, two normally silent subtelomeric *EPA* genes (*EPA6* and *EPA7*) are transcribed and contribute strongly to the overall adherence of the mutant strain (*8*). To understand the role of chromatin silencing in the regulation of *C. glabrata* adherence and virulence, we asked specifically whether *EPA6* is normally transcribed during the course of an infection.

To assess *EPA6* expression during the course of animal infections, we used a recombinational in vivo expression technology approach (*9*, *10*). We constructed a reporter strain, BG1087, in which we replaced the chromosomal *EPA6* open reading frame (ORF) with the *S. cerevisiae FLP1* ORF encoding the Flp1p site-specific recombinase. In this same strain, we modified the *TRP1* locus by placing the *PGK1* promoter upstream of the *TRP1* coding region, followed by a promoterless *hph* gene from *Klebsiella pneumoniae* [encoding hygromycin B resistance (Hyg^R)]. We engineered *FRT* sites flanking the *TRP1* gene (Fig. 1A). Flp1-mediated recombination results in loss of the *TRP1* ORF, rendering the cells Trp$^-$ Hyg^R, in contrast to the Trp$^+$ Hyg-sensitive (Hyg^S) parent strain. Consistent with the low level of *EPA6* expression, we found that only 1% of cells were Hyg^R in cultures of the *EPA6::FLP1* grown for 40 generations in vitro; as expected, if the *EPA6*::*FLP1* cassette was carried on a plasmid (where it is not subject to silencing), the entire population was Hyg^R by the time cultures could be analyzed (*11*).

We used this system to study the expression of *EPA6* in two infection models. First, mice were infected intravenously with strain BG1087, and yeast were recovered from target organs 4 and 7 days after infection. There was no increase in the percentage of Hyg^R colonies recovered from target organs as compared with the percentage present in the initial inoculum (Fig. 1B). We also adapted an established murine model of UTIs (*12*) to study *C. glabrata* UTI. After delivery of an inoculum transurethrally, the bladder was colonized and infection ultimately ascended to the kidney (*11*). In this model, nearly all mice infected with strain BG1087 showed a significant increase in the percentage of Hyg^R colonies recovered from both the bladder and kidney (Fig. 1B). These data suggest that *EPA6* is transcribed during UTI but not during disseminated bloodstream infection. To test the hypothesis that urine might induce *EPA6* expression, we used a strain in which green fluorescent protein (GFP) replaces the *EPA6* ORF at the normal chromosomal locus; as expected, *EPA6*::GFP was silenced in a wild-type background but was strongly transcribed in a *sir3*Δ background (fig. S1). Expression of *EPA6*::GFP or of the *EPA6* gene itself was induced when the strain was grown in human urine samples (Fig. 2A and fig. S2). In order to determine what components of urine might be important for this induction, we examined *EPA6* induction in a defined synthetic urine medium (*13*). This is essentially a nutritionally poor medium [5% synthetic complete (SC)] supplemented with urine-specific salts and urea (*14*). *EPA6*::GFP expression was induced in synthetic urine, and this induction was independent of urine-specific components but occurred simply in 5% SC (fig. S3A) as a result of limitation of the vitamin niacin [or nicotinic acid (NA)] (Fig. 2B). Limitation of other components of SC did not induce *EPA6::GFP* transcription (fig. S3, B and C). These results were confirmed by direct measurement of *EPA6* transcript levels (fig. S3D).

Because NA limitation induced *EPA6* expression, we examined the NA requirements for *C. glabrata* growth and found that *C. glabrata* is an NA auxotroph (Fig. 3A). The closely related *S. cerevisiae* is an NA prototroph because it can synthesize nicotinic acid mononucleotide (NaMN) from tryptophan via the kynurenine pathway [the enzymes of which are encoded by the *BNA* genes (*15*)] (fig. S4). Inspection of the recently published *C. glabrata* genomic sequence (*16*) showed that *C. glabrata* is lacking all of the functional *BNA* genes (fig. S5). *C. glabrata* is also auxotrophic for thiamine and pyridoxine (fig. S6A), but equivalent growth limitation of *C. glabrata* by limiting

[1]Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA. [2]Division of Infectious Diseases, [3]Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA. [4]Research Service, Department of Veterans Affairs, Baltimore, MD 21201, USA.

*Present address: Instituto Potosino de Investigacion Cientifica y Tecnologica, Division de Biologia Molecular, Camino a la Presa San Jose 2055, 78216 San Luis Potosi, San Luis Potosi, Mexico.

†To whom correspondence should be addressed. E-mail: bcormack@jhmi.edu

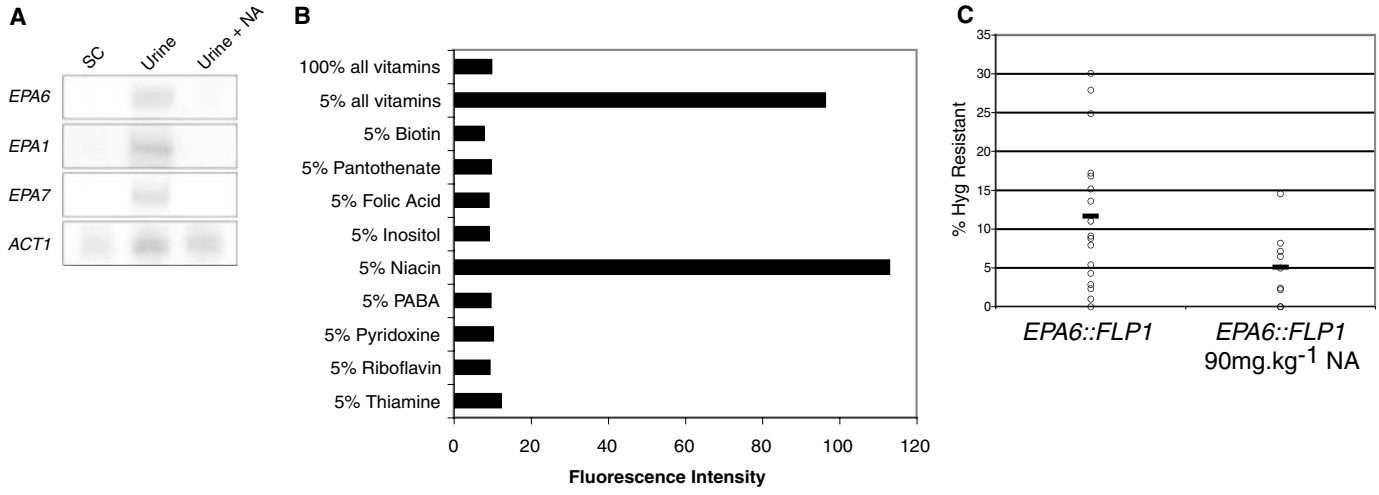
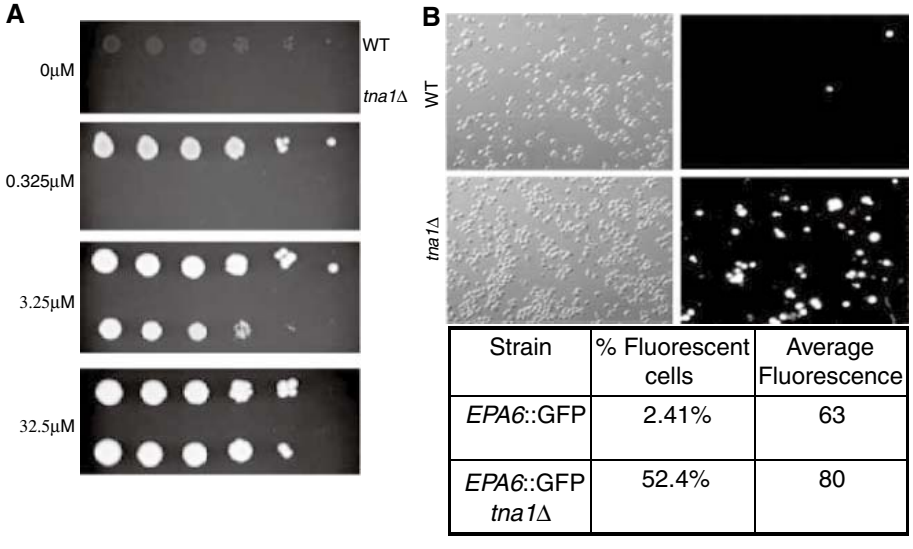


Fig. 2. Transcriptional induction of *EPA6* during growth in urine or NA-limited media. (**A**) S1 nuclease protection assay of *EPA1*, *EPA6*, and *EPA7* transcripts in strain BG2 (wild-type) grown in human urine with or without supplemental NA. (**B**) Fluorescence of strain BG1045 (*EPA6*::GFP) cultured in SC media limited (5% of normal) in individual vitamins. (**C**) Hyg^R phenotype (expressed as a percentage of total cells recovered) of strain BG1087 recovered from a mouse bladder 4 days after infection. Each symbol (circles) corresponds to yeast recovered from a single animal. Results are shown for infections of mice fed normal diets or supplemented daily with NA (90 mg/kg). Bars represent the mean.

NA, thiamine, or pyridoxine showed that only NA limitation resulted in induction of *EPA6* (fig. S6B).

After demonstrating that NA limitation in yeast medium or in synthetic urine cultures resulted in *EPA6* induction, we confirmed that *EPA6* induction in urine itself was also the result of NA limitation, because addition of excess NA or the related compound nicotinamide (NAM) suppressed the induction of *EPA6* (Fig. 2A and fig. S2). To test whether NA limitation contributed to *EPA6* induction during experimental UTI, we tested whether alteration of NA levels in the urine of mice could change the induction of *EPA6* during infection. Rodents fed an excess of NA excrete the excess NA in the form of NA and NAM [as well as various metabolites such as nicotinuric acid and methyl nicotinamide (*17*)]. In our laboratory, mice fed a diet supplemented daily with 90 mg of NA per kg of feed (mg/kg) (versus approximately 15 mg/kg in daily consumption of normal mouse feed) had combined urine levels of NA and NAM about five times higher than in mice kept on a control diet (*14*). When we infected these mice with the *EPA6::FLP1* strain, we found that, on average, 5% of the cells recovered from the bladders of mice fed the high-NA diet were Hyg^R, compared with 12% of cells recovered from the bladders of mice fed a normal diet (Fig. 2C). This difference was marginally significant ($P = 0.055$) and consistent with a role for NA limitation during UTI, contributing to induction of *EPA6*.

Because *C. glabrata* is an NA auxotroph, it relies solely on exogenous sources of NA for growth. In *S. cerevisiae*, NA is transported from the environment by the high-affinity transporter Tna1p (*18*). We deleted the *C. glabrata TNA1* ortholog in the *EPA6*::GFP reporter strain to measure the effect on *EPA6* induction. The *tna1*Δ *EPA6*::GFP strain cannot grow at concentrations of NA sufficient for growth of the wild-type strain (Fig. 3A), which is consistent with a role in high-affinity NA transport. For cells growing in SC media (3.25 μM NA), *EPA6::*GFP was essentially not expressed in wild-type cells, but it was expressed in the majority of *tna1*Δ cells (Fig. 3B), consistent with a decrease in



Strain	% Fluorescent cells	Average Fluorescence
EPA6::GFP	2.41%	63
EPA6::GFP *tna1*Δ	52.4%	80

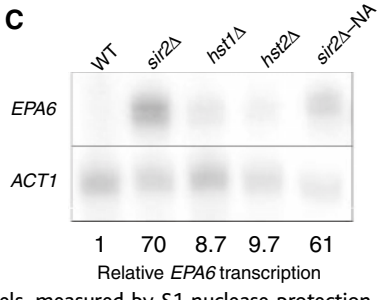


Fig. 3. Growth and silencing in *C. glabrata tna1*Δ, *sir2*Δ, *hst1*Δ, and *hst2*Δ strains. (**A**) Strain BG1045 and strain BG1176 grown on SC NA plates supplemented with a range of NA concentrations (from 0.325 to 32.5 μM). WT, wild type. (**B**) Phase-contrast and fluorescence microscopy showing *EPA6*::GFP fluorescence of strains BG1045 and BG1176 grown in SC (3.25 μM NA). The table below shows fluorescence-activated cell sorter (FACS) quantitation of cells grown on SC (3.25 μM NA) plates. The percent of all cells that are fluorescent is shown, as well as the average fluorescence intensity of those cells that are fluorescent. (**C**) *EPA6* transcript levels, measured by S1 nuclease protection, in strains BG2 (WT), BG1048 (*sir2*Δ), BG1073 (*hst1*Δ), and BG1112 (*hst2*Δ). Also indicated is the fold increase of the *EPA6* transcript (normalized to *ACT1* levels) relative to levels in BG2.

A % Adherence

	Strain	T24	A498
SC	WT	1.9±0.005	4.2±0.41
	*epa1*Δ	1.8±0.03	4.7±0.71
	*epa(1,6,7)*Δ	0.56±0.22	3.69±2.1
SC-NA	WT	19.6±0.85	25.9±1.3
	*epa1*Δ	10.2±1.3	12.0±0.61
	*epa(1,6,7)*Δ	0.73±0.035	4.1±0.89
Urine	WT	10.3±0.56	17.0±0.029
	*epa1*Δ	7.2±0.59	15.1±0.82
	*epa(1,6,7)*Δ	1.06±0.48	1. 3±0.20
Urine + 100xNA	WT	1.82±0.085	3.7±1.3
	*epa1*Δ	2.75±0.13	3.9±0.058
	*epa(1,6,7)*Δ	1.25±0.35	1.4±0.052

B

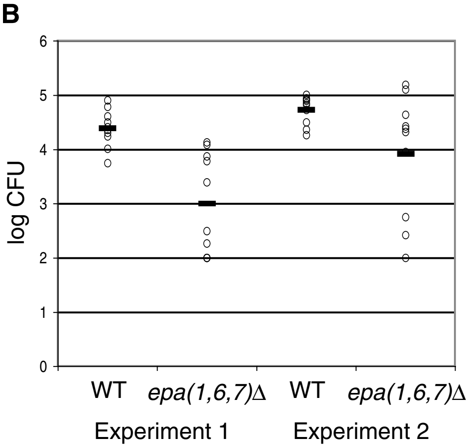


Fig. 4. Adherence of *C. glabrata* cells grown in urine. (**A**) Adherence to uroepithelial T24 and A498 cells of *C. glabrata* strains BG2 (WT), BG178 (*epa1*Δ), and BG1026 [*epa(1,6,7)*Δ] grown in SC; SC with limited NA; urine; or urine supplemented with NA, expressed as the percent of adherent cells present in each culture. (**B**) Colonization of murine bladder by strains BG462 (WT) and BG1026 [*epa(1,6,7)*Δ]. Circles represent the colony-forming units (CFU) recovered from individual animals and bars represent the geometric mean.

cellular NA levels signaling a loss of *EPA6* silencing.

NA is a precursor of nicotinamide adenine dinucleotide (NAD^+), and our results suggested that limiting environmental NA levels leads to reduced intracellular NAD^+ levels, thus affecting Sir2p NAD^+- dependent histone deacetylase activity and ultimately, *EPA6* silencing. To test this model, we examined the role of *SIR2* and two related NAD^+-dependent histone deacetylases, encoded by the *HST1* and *HST2* genes, which have been implicated in *S. cerevisiae* in repression of transcription (*7*, *19*, *20*). We deleted the *C. glabrata* orthologs of *SIR2*, *HST1*, and *HST2* and assessed the derepression of *EPA6* in each background. *EPA6* was derepressed most significantly in the *sir2*Δ background (Fig. 3C); moreover, there was no further increase in *EPA6* expression when the *sir2*Δ strain was grown under NA-limited conditions, consistent with NA limitation signaling *EPA6* derepression via its effect on Sir2p. If NA limitation induces *EPA6* via Sir2p, then multiple genes regulated by subtelomeric silencing should be derepressed under those growth conditions. We found that three telomeric *EPA* genes—*EPA1*, *EPA6*, and *EPA7*—were induced by growth in urine and that this induction could be suppressed by the addition of excess NA (Fig. 2A). We also saw a modest increase in the levels of *EPA4* and *EPA5* (*11*). This pattern of *EPA* gene derepression was essentially identical to that seen in strains in which *SIR3* was deleted (*8*) and suggests that NA-limited growth results in a general loss of silencing, mimicking the absence of *SIR3*.

Because at least some of the *EPA* genes are adhesins (*8*), we assessed whether growth in urine had any impact on in vitro adherence to uroepithelial cells. We assessed adherence to T24 cells (human bladder epithelial cells) and A498 cells (human proximal tubular renal epithelial cells) and found that growth of wild-type and *epa1*Δ cells in urine or NA-limited medium increased adherence to both cell types. By contrast, an *epa(1,6,7)*Δ strain did not adhere significantly to either cell type (Fig. 4A), suggesting that the three *EPA* genes that were induced by growth in urine also mediate most of the uroepithelial adherence induced by NA limitation. These data were mirrored by experiments with a *sir3*Δ strain (table S1). Lastly, we found that the *epa(1,6,7)*Δ strain showed a decreased (P = 0.018) ability to colonize the bladder in the murine UTI model when compared to the wild-type parent strain, consistent with a functional role for *EPA* genes in colonization of the urinary tract (Fig. 4B).

It has been hypothesized that changes in cell metabolism that alter cellular NAD^+ levels might modulate Sir2p activity (*21*, *22*). Here we provide data suggesting that cellular NAD^+ levels, which in *C. glabrata* are tied to environmental NA levels, modulate Sir2p-mediated silencing of subtelomeric *EPA* genes in response to the host environment. The cellular responses of *C. glabrata* and *S. cerevisiae* to NA limitation differ markedly. NA limitation in *S. cerevisiae* does not result in loss of silencing (*22*), because the de novo NAD^+ biosynthesis pathway (encoded by the *BNA* genes) acts to maintain a constant cellular NAD^+ level independent of environmental NA (*23*). Inspection of genome sequences (http://seq.yeastgenome.org/cgi-bin/nph-blast-fungal.pl) showed that the *BNA* pathway is broadly distributed among fungi (being present, for example, in *C. albicans*, *Neurospora crassa*, *Aspergillus nidulans*, and *Cryptococcus neoformans*). *C. glabrata* has deleted all the *BNA* genes [with the exception of *BNA3* (*24*)], which is striking because the *S. cerevisiae* and *C. glabrata* genomes are highly related; of the nearly 6000 *S. cerevisiae* ORFs, only approximately 200 have no obvious ortholog in *C. glabrata* (*25*). The *BNA* genes are present in *Eremothecium* (http://agd.unibas.ch) and *Kluyveromyces* (www.broad.mit.edu/seq/YeastDuplication/) in blocks that are syntenic relative to the *S. cerevisiae* genome, consistent with the pathway having been lost in the *C. glabrata* lineage rather than recently acquired in the *S. cerevisiae* lineage.

That *C. glabrata* is an NA auxotroph suggests that there must be microbiologically available NA in *C. glabrata*'s ecological niche. *C. glabrata* is not normally isolated from the environment but is found only in association with the mammalian host (*26*). Relevant to the experiments in this paper, urine is known to contain both NA and NAM (*27*). Urinary tract isolates of *Escherichia coli* are also often NA auxotrophs (*28*, *29*). Our data also imply that the NA levels sensed by *C. glabrata* in the urinary tract are sufficient to support growth but insufficient for full Sir2p function.

What is the impact of NA limitation on the virulence of *C. glabrata*? NA limitation induces three adhesins—*EPA1, EPA6*, and *EPA7*—which we show have roles in adherence to uroepithelial cells in vitro and in bladder colonization in the mouse UTI model. For *C. glabrata*, and indeed *C. albicans*, colonization of the urinary tract is seen in the hospital setting primarily in the context of indwelling catheters on which *Candida* can form biofilms. *EPA6* has recently been implicated in biofilm formation in vitro (*30*). Perhaps NA limitation in the urinary tract, or specifically on catheter surfaces, might increase *EPA6* expression and stimulate the formation of catheter-associated biofilms.

We suggest that *C. glabrata*'s evolutionary adaptation to the host included two sets of events: the loss of the *BNA* genes (and resulting NA auxotrophy) and the encoding of the *EPA* adhesin family at subtelomeric loci. We provide evidence that these aspects of the *C. glabrata* genome, combined with the NAD^+ dependence of Sir2p, are key features governing the regulation of the *EPA* genes in response to particular NA-limited host environments.

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A Synaptonemal Complex Protein Promotes Homology-Independent Centromere Coupling

Tomomi Tsubouchi[1] and G. Shirleen Roeder[1,2*]

We describe a process in meiotic cells of budding yeast in which chromosomes become joined together in pairs at their centromeres independent of chromosomal homology. These centromeric interactions depend on the synaptonemal complex component Zip1. During meiosis in wild-type diploids, centromere couples are initially nonhomologous and then undergo switching until all couples involve homologs. This transition to homologous coupling depends on Spo11, a protein required for the initiation of meiotic recombination. Regions of synaptonemal complex assembled early in meiosis are often centromere-associated. We propose that centromere coupling facilitates homolog pairing and promotes synapsis initiation.

Segregation of chromosomes at the reductional division of meiosis depends on a series of interactions between homologous chromosomes, including pairing, assembly of the synaptonemal complex (SC), genetic recombination, and formation of chiasmata. Pairing relies on both recombination-independent and recombination-dependent mechanisms. Although recombination is required for full levels of pairing, a substantial amount of homolog alignment occurs in the absence of recombination (*1*). Recombination-independent pairing is believed to depend, at least in part, on telomere clustering leading to bouquet formation (*2*, *3*).

The Zip1 protein is a component of the SC in budding yeast. Zip1 is a coiled-coil protein that bridges the space between the cores of homologous chromosomes (*4*). Although formation of the SC serves to stabilize pairing interactions between homologs, the complex is generally not assumed to play a role in pairing per se.

Meiotic recombination initiates with the formation of developmentally programmed double-strand breaks in the DNA (*5*). In budding yeast, mutants defective in the initiation of meiotic recombination, such as *spo11*, fail to make mature SCs (*1*). However, the Zip1 protein does localize to discrete foci on chromosomes in a *spo11* background, as detected by immunostaining of surface-spread meiotic nuclei (Fig. 1A) (*6*, *7*). Unlike the polymerization of Zip1 along the lengths of chromosomes (*8*, *9*), the localization of Zip1 to foci in *spo11* strains is independent of the Zip2 and Zip3 proteins (*10*), components of the synapsis initiation complex (*11*). Formation of Zip1 foci in *spo11* also does not require the meiosis-specific chromosomal core protein Red1 (*10*, *12*).

We found that the chromosomal Zip1 foci present in *spo11* strains are often located at or near centromeres. Figure 1A shows a meiotic nucleus stained with antibodies to both Zip1 and an epitope tag fused to Ctf19, a component of the yeast kinetochore (*6*, *13*). Most Zip1 foci (78.1 ± 11.7%) overlap with Ctf19 foci (47 nuclei scored).

Almost all nuclei from *spo11* strains contain a polycomplex, which is an aggregate of Zip1 proteins unassociated with DNA (*1*). Ctf19 and the centromere protein Ndc10 are often found in the polycomplex (Fig. 1 and fig. S1), indicating that the interaction between these proteins and Zip1 does not require an intact centromere.

A diploid yeast cell contains 32 chromosomes representing 16 pairs of homologs. The number of centromere (i.e., Ctf19) foci per meiotic prophase nucleus averages 17 in the *spo11* mutant and shows little variation among nuclei (Fig. 1B). Because the number of centromere foci is about half the number of chromosomes, these observations suggest that centromeres become joined together in groups of two. This association will hereafter be referred to as "coupling" or "centromere coupling," although we cannot distinguish between coupling at the centromere per se versus coupling involving centromere-proximal sequences.

In budding yeast, the Ndj1 protein is required for bouquet formation and efficient homolog pairing (*3*). To determine whether Ndj1 is important for centromere coupling, we measured the number of Ctf19 foci in diploid nuclei from an *ndj1 spo11* double mutant (Fig. 1D). The average number of centromere foci is 15, indicating that centromere coupling is not dependent on bouquet formation.

Does centromere coupling require the Zip1 protein? In a *zip1 spo11* double mutant, the number of foci increased to about 32 (Fig. 1C), indicating that centromeres are not coupled in the absence of Zip1.

The observation that there are ~16 centromere foci per nucleus suggested that the centromeres of homologous chromosomes are paired in a *spo11* background. To test this hypothesis, we used the *lacO*/LacI–green fluorescent protein (GFP) system (*14*)

[1]Department of Molecular, Cellular, and Developmental Biology, [2]Department of Genetics and Howard Hughes Medical Institute, Yale University, New Haven, CT 06520, USA.

*To whom correspondence should be addressed. E-mail: shirleen.roeder@yale.edu

to visualize centromere-proximal regions of a homolog pair (either chromosome III or XI) (*6*). The centromeres of homologous chromosomes are unpaired in most nuclei (Fig. 2, A and B). The frequency of pairing is 23.9% for *CENIII* and 15.3% for *CENXI*. Thus, the majority of centromere couples in a *spo11* strain represent interactions between the centromeres of two nonhomologous chromosomes.

To obtain independent evidence of nonhomologous centromere coupling, we counted centromere foci in a *spo11* haploid strain engineered to enter meiosis (*6*). Such a strain contains 16 chromosomes with no potential for homolog pairing. In a *spo11* haploid, the average number of Ctf19 foci per nucleus was eight (Fig. 2C), indicating that nonhomologous chromosomes are coupled. As is the case in diploids, the Zip1 protein colocalizes with centromeres in *spo11* haploid cells (87.2 ± 11.0% of Zip1 foci colocalize with Ctf19 foci; 48 nuclei scored), and centromere coupling requires the Zip1 protein (Fig. 2D). We observed about eight centromeric foci in nuclei from a wild-type haploid (Fig. 2E) and 16.3 ± 1.8 foci in a *zip1* haploid (37 nuclei scored), demonstrating that Zip1-dependent coupling of nonhomologous centromeres is not unique to the *spo11* mutant (*10*).

To determine whether Zip1-mediated centromere coupling takes place during normal meiosis, we examined centromere coupling throughout meiosis in wild-type diploids. On the basis of the Zip1 staining pattern, nuclei were classified into three categories, I, II, and III, representing progressively later stages in SC assembly (*6*). Nuclei in category I are similar to *spo11* nuclei in terms of the number of Ctf19 foci (Fig. 3D), the extent of Zip1 colocalization with centromeres (Fig. 3A), and the degree of homologous centromere pairing (Fig. 3G). As synapsis progresses, the number of Ctf19 foci remains fairly constant (Fig. 3, D to F), whereas the frequency of homologous centromere coupling increases until full pairing is achieved (Fig. 3, G to I).

In category II nuclei, linear stretches of Zip1 are often associated with a centromere (Fig. 3B), suggesting that synapsis (i.e., the elongation of Zip1 along chromosomes) initiates at centromeres. To quantitate this association, we focused our attention on short linear stretches of Zip1 staining in nuclei at early stages of synapsis initiation (Fig. 4) (*6*). Of 129 linear stretches examined, 98 (76%) were associated with a centromere.

Our data demonstrate that meiotic chromosomes become joined together in pairs at their centromeres and that this coupling is both dynamic and independent of chromosomal homology. In wild-type cells, the number of

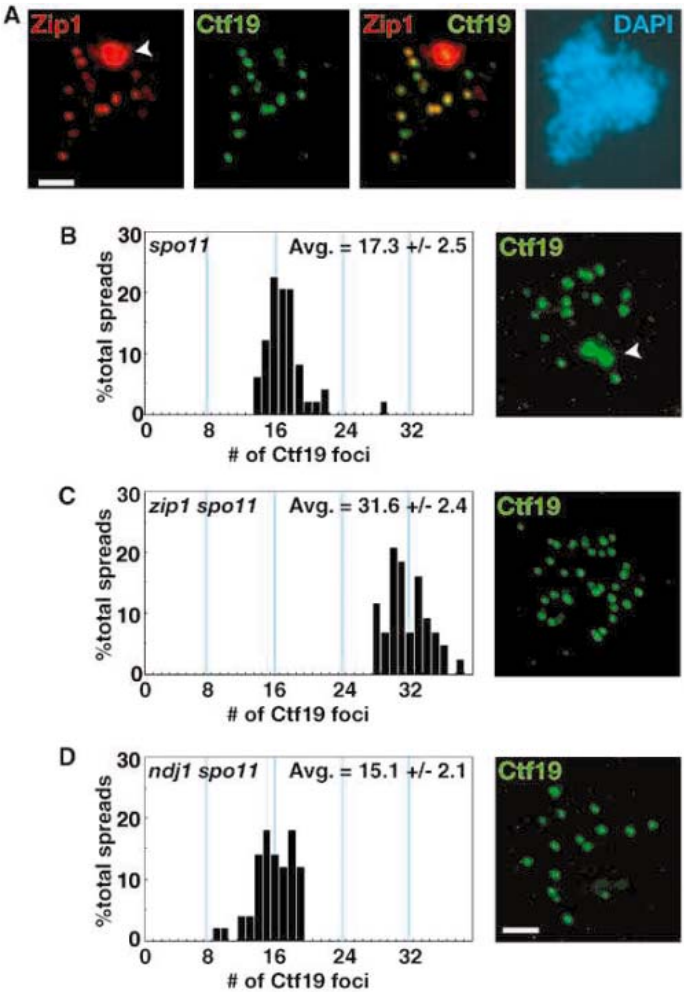

Fig. 1. Centromeres are coupled in a Zip1-dependent manner in the *spo11* mutant. (**A**) A single meiotic nucleus that has been surface-spread and stained for Zip1 and Ctf19-myc and with the DNA-binding dye 4′,6′-diamidino-2-phenylindole (DAPI). Quantification of Ctf19 foci in (**B**) *spo11*, (**C**) *zip1 spo11*, (**D**) and *ndj1 spo11* diploids. A representative Ctf19-stained nucleus is shown for each strain. Indicated in each graph is the average (Avg.) number of Ctf19 foci with standard deviation. Numbers of nuclei scored are, for *spo11*, 49; *zip1 spo11*, 45; and *ndj1 spo11*, 46. Arrowheads indicate the positions of polycomplexes. Bars indicate 2 μm.

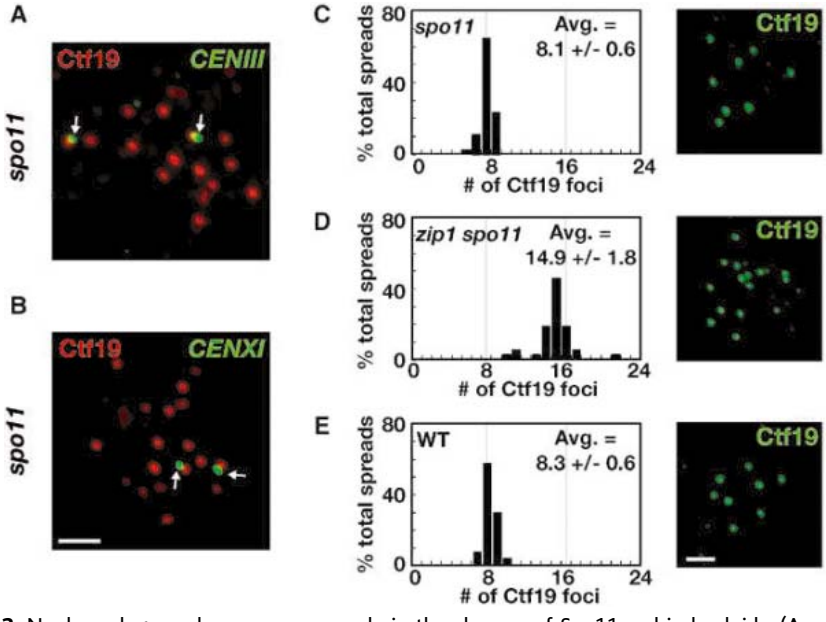

Fig. 2. Nonhomologous chromosomes couple in the absence of Spo11 and in haploids. (**A** and **B**) Nonhomologous centromere coupling in *spo11*. Diploid *spo11* cells with *LacO* insertions near (A) *CENIII* or (B) *CENXI* were spread and stained for LacI-GFP and Ctf19-myc. Arrows point to the locations of homologous chromosomes. Numbers of nuclei scored: *CENIII*, 88, and *CENXI*, 59. Quantification of Ctff19 foci in (**C**) *spo11*, (**D**) *zip1 spo11*, and (**E**) wild-type (WT) haploids. Representative Ctf19-stained nuclei are shown. Indicated in each graph is the average number of Ctf19 foci with standard deviation. Numbers of nuclei scored: *spo11*, 56; *zip1 spo11*, 38; and WT, 50. Bars, 2 μm.

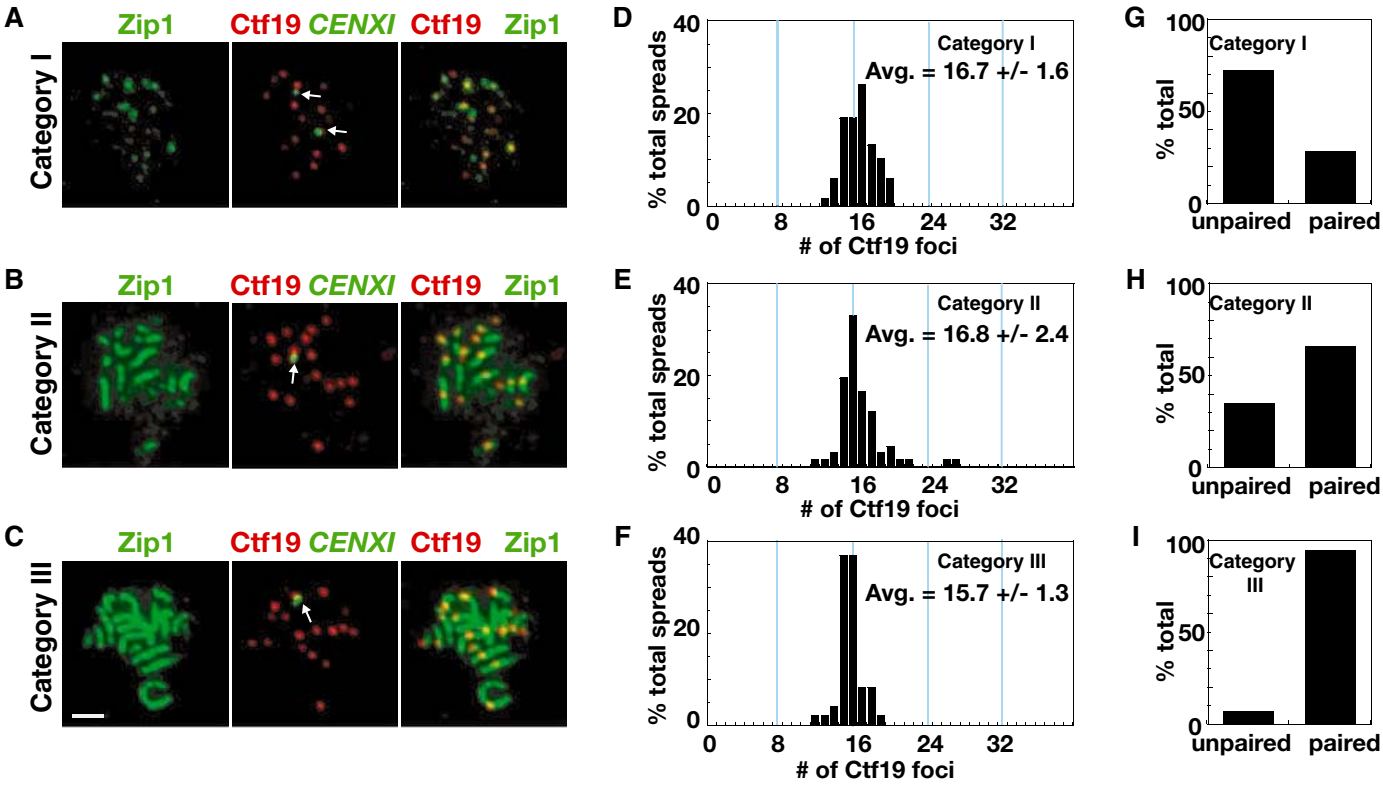

Fig. 3. Chromosome coupling transitions from nonhomologous to homologous in wild-type. (**A** to **C**) Wild-type cells with *LacO* insertions near *CENXI* were spread and stained for Zip1, Ctf19-myc, and LacI-GFP. The arrows indicate *CENXI* locations. Examples of nuclei with (A) unpaired and (B and C) paired *CENXI* are shown. Quantification of Ctf19 foci in nuclei of categories I (**D**), II (**E**), and III (**F**). Representative Ctf19-stained nuclei are shown. Indicated in each graph is the average number of Ctf19 foci with standard deviation. Quantification of *CENXI* pairing in nuclei of categories I (**G**), II (**H**), and III (**I**). The number of nuclei used to measure Ctf19 foci and *CENXI* pairing was 69, 67, and 49 for categories I, II, and III, respectively. Bar, 2 μm.

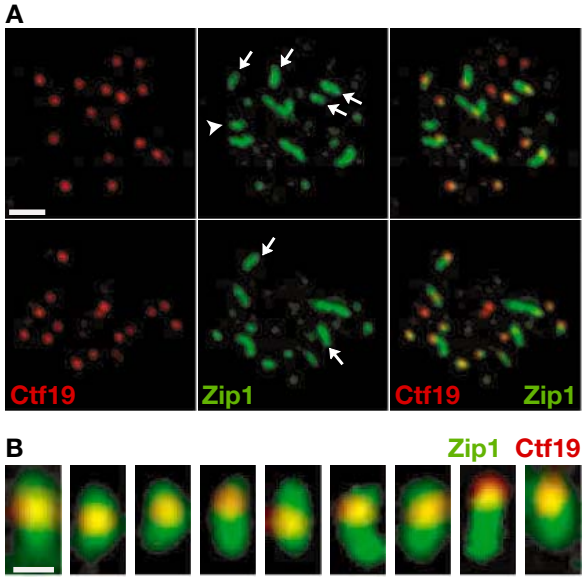

Fig. 4. Early synapsis initiates at centromeres. (**A**) Examples of nuclei at early stages of synapsis initiation. Wild-type spreads were stained for Zip1 and Ctf19-myc. Arrows and an arrowhead indicate Zip1 stretches used to quantitate centromere associations. Of the seven stretches shown, only one (arrowhead) is not associated with a centromere. Bar, 2 μm. (**B**) Additional examples of Zip1 stretches used to measure the frequency of synapsis initiation at centromeres. Bar, 0.5 μm.

centromere couples (i.e., Ctf19 foci) remains fairly constant throughout meiotic prophase, whereas the fraction of homologous couples steadily increases. Thus, centromeres that become uncoupled must immediately form new associations. Perhaps dissociation of an existing couple is triggered only after a new partner is recognized.

What function(s) might Zip1-mediated centromere coupling perform? We propose that coupling facilitates homolog pairing by holding two chromosomes together in a stable configuration while homology is being assessed. However, centromere coupling is not essential for pairing, because homologs do align correctly in a *zip1* mutant (*15*). Centromere coupling, bouquet formation, and recombination might all contribute in different ways to the efficacy of pairing.

The transition from largely nonhomologous centromere coupling to homologous coupling during meiotic prophase depends on the Spo11 protein, presumably through its role in the initiation of recombination (*5*). Coupling occurs in haploids even when Spo11 is present, indicating that Spo11 does not function to prevent associations between nonhomologous chromosomes. Instead, Spo11 probably plays a role in the recognition of homologous partners. Once two chromosomes are recognized as homologs, they may become irreversibly locked together at their centromeres and thus excluded from subsequent switching events.

Our data strongly suggest that homologously coupled centromeres serve as sites of synapsis initiation. Homolog recognition may trigger the polymerization of Zip1 at centromeres, providing the locking mechanism proposed above. Previous studies (of pachytene nuclei) have indicated that synapsis initiates at noncentromeric locations (*8*, *9*, *11*). These observations can be easily reconciled if one supposes that synapsis initiates predominantly at centromeres at early times, but noncentromeric sites make an increasingly important

contribution to synapsis initiation as meiosis progresses.

Another function for centromere coupling might be the distributive disjunction system, which ensures the segregation of chromosomes that failed to cross over. Centromere regions in female flies have been shown to be important for distributive disjunction (*16*). In yeast, nonexchange chromosomes associate at their centromeres before segregation, and interfering with this association increases the rate of nondisjunction (*17*).

Centromeres appear to contribute to homolog alignment and segregation in other species as well, although the underlying mechanisms may be somewhat different. Nonhomologous centromere coupling is observed before homolog pairing during meiosis in wheat, but it is also observed in premeiotic tissues, arguing that centromere coupling does not depend on an SC protein (*18*, *19*). In fission yeast, only centromeric regions are able to pair independently of meiotic recombination (*20*). The contributions of centromeres and SC proteins to homolog pairing in higher eukaryotes remain to be elucidated.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/308/5723/870/DC1
Materials and Methods
Fig. S1
References

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Attractor Dynamics in the Hippocampal Representation of the Local Environment

Tom J. Wills,[1] Colin Lever,[1] Francesca Cacucci,[1] Neil Burgess,[1,2] John O'Keefe[1]*

Memories are thought to be attractor states of neuronal representations, with the hippocampus a likely substrate for context-dependent episodic memories. However, such states have not been directly observed. For example, the hippocampal place cell representation of location was previously found to respond continuously to changes in environmental shape alone. We report that exposure to novel square and circular environments made of different materials creates attractor representations for both shapes: Place cells abruptly and simultaneously switch between representations as environmental shape changes incrementally. This enables study of attractor dynamics in a cognitive representation and may correspond to the formation of distinct contexts in context-dependent memory.

For more than 30 years, autoassociative or attractor dynamics based on Hebbian synaptic modification have been central to neuronal models of memory, with particular focus on the dense recurrent collaterals of the hippocampus and its crucial role in context-dependent episodic memory (*1–14*). We investigated whether attractors were present in

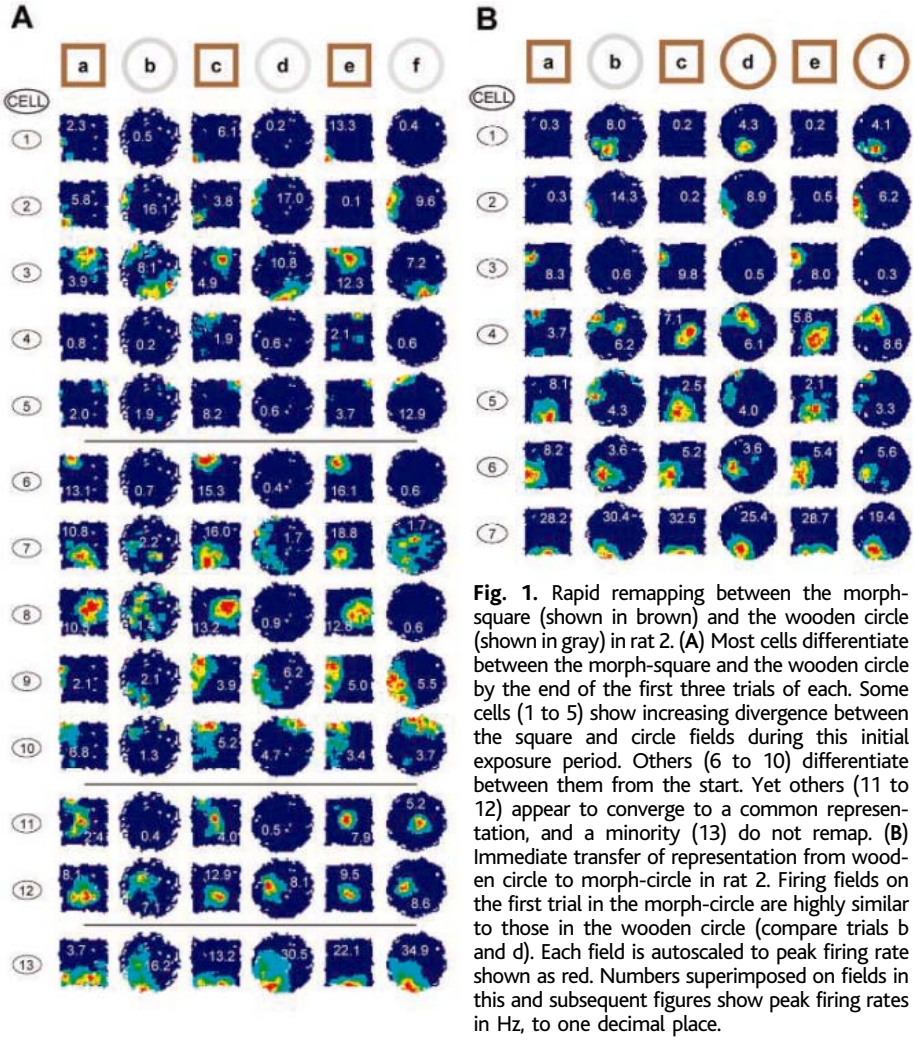

Fig. 1. Rapid remapping between the morph-square (shown in brown) and the wooden circle (shown in gray) in rat 2. (**A**) Most cells differentiate between the morph-square and the wooden circle by the end of the first three trials of each. Some cells (1 to 5) show increasing divergence between the square and circle fields during this initial exposure period. Others (6 to 10) differentiate between them from the start. Yet others (11 to 12) appear to converge to a common representation, and a minority (13) do not remap. (**B**) Immediate transfer of representation from wooden circle to morph-circle in rat 2. Firing fields on the first trial in the morph-circle are highly similar to those in the wooden circle (compare trials b and d). Each field is autoscaled to peak firing rate shown as red. Numbers superimposed on fields in this and subsequent figures show peak firing rates in Hz, to one decimal place.

the hippocampal representations of different environments.

Place cells in the mammalian hippocampus signal the location of the animal within its environment by firing whenever it visits a specific region [the "place field" (*10*, *15*)]. This representation can be specific to the environment, with different cells being active in different environments or the same cell being active at different locations in different environments (*16*). The change in representation between environments is known as "remapping." After foraging in square and circular boxes which differed only in their shapes (not texture or color), CA1 hippocampal place cells took considerable time (many days or weeks) to differentiate between the two boxes, with simultaneously recorded cells remapping at different times (*17*). Individual cells appeared to represent a location in one or both environments independently of other cells.

We recorded from CA1 place cells in a paradigm designed to produce more rapid remapping (*18*). Animals were initially exposed to a square and a circle that differed in color, texture, and shape. The square was a morph-box (*17*) (which can be configured in various shapes, see fig. S1, A and B); the circle was made of painted wood. This led to rapid remapping with the majority of cells (92%, that is, 48 out of 52) differentiating between the environments at the end of the first day's six trials (three in each box, see Fig. 1A). After 3 days of this training, the animals were trained in the morph-box configured as a square and a circle on alternate trials for an additional 3 days (fig. S1C). The place fields of the majority of remapped cells (40 out of 46) transferred successfully to the morph-circle and showed the same pattern as in the wooden circle (see Fig. 1B). Different place fields in two configurations of the morph-box can only be cued by environmental shape, as other attributes such as texture and color do not vary. Of the six animals, one failed to show rapid remapping in the morph-square and wooden circle, and one did not show wooden circle to morph-circle pattern transfer. In these cases, the experiment was terminated. This paper describes results from the remaining four animals.

Are the different hippocampal representations of the morph-square and morph-circle after remapping due to the formation of separate attractors for each shape? If so, each representation would lie at the bottom of a "basin of attraction" within which other representations inevitably evolve into the attractor representation under the system's dynamics: Representations of intermediate shapes would revert to either the square or the circle representation (*3*, *6*) (fig. S3, A to C). If not, representations of intermediate shapes would remain intermediate to those of the circle and square.

We recorded from groups of neurons during a series of probe trials in a set of octagonal morph-boxes (*18*) that varied from squarelike (adjacent side ratio 1:7) to circlelike (adjacent side ratio 4:4) through more ambiguous intermediates (see Fig. 2 top row; fig. S1D). Almost all simultaneously recorded cells (28 out of 33) showed an abrupt switch from the squarelike pattern to the circlelike one across the octagonal series. The firing fields of 20 simultaneously recorded place cells in the series are shown in Fig. 2. Trials are presented in order of most squarelike on the left to most

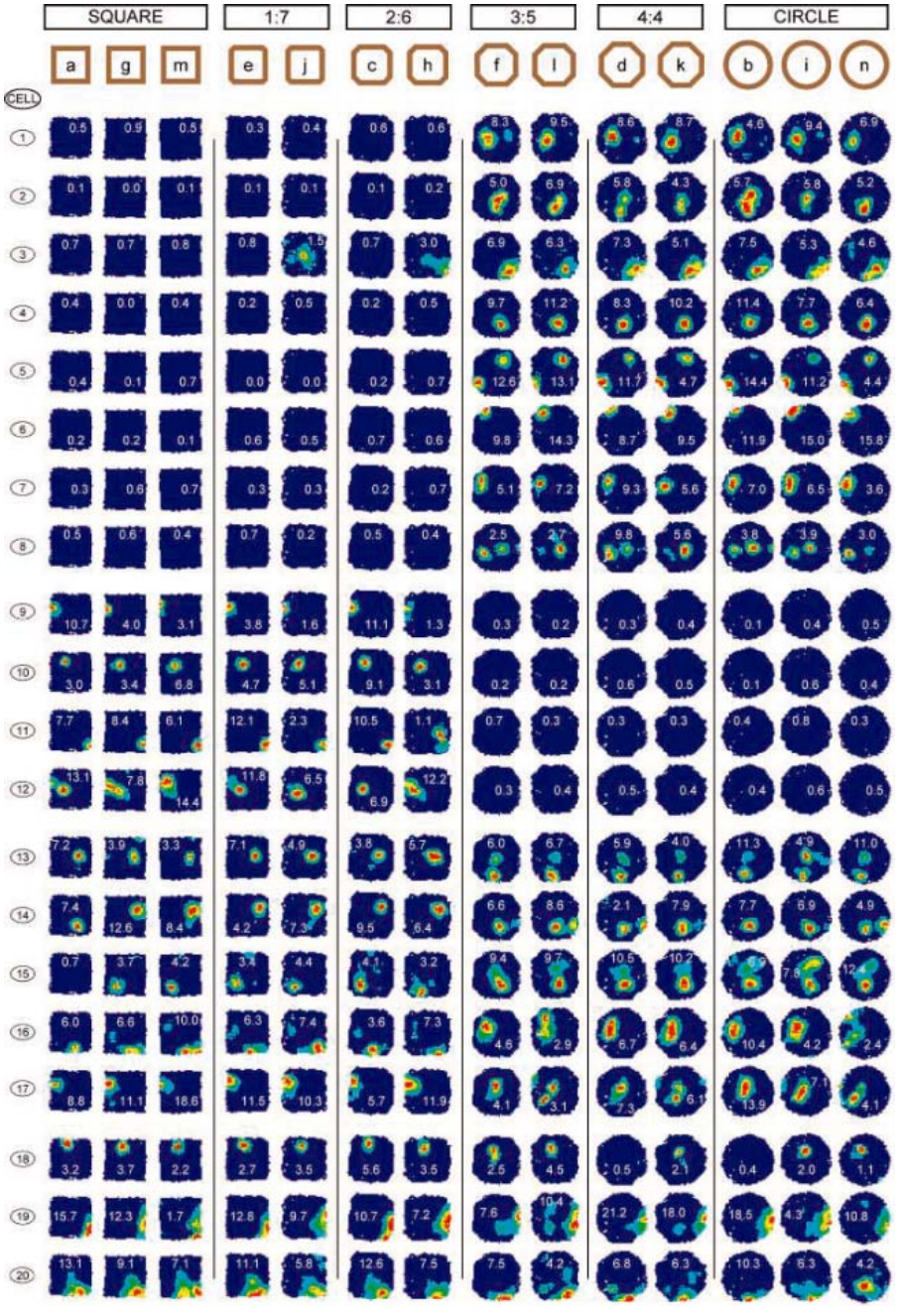

Fig. 2. Abrupt and coherent expression of squarelike or circlelike representation during probe trials in intermediate octagonal environments in rat 4. The 17 of 20 place cells simultaneously recorded from rat 4 with different (remapped) firing patterns in the square and the circle almost all switch from the squarelike to circlelike pattern between the 2:6 and 3:5 octagons. Eight cells had fields in the circle but not the square (cells 1 to 8); four in the square but not the circle (9 to 12); five fired in both but in different places (13 to 17); and three did not reach our criterion for remapping (18 to 20) (*18*).

[1]Department of Anatomy and Developmental Biology, [2]Institute of Cognitive Neuroscience, University College London, London WC1E 6BT, UK.

*To whom correspondence should be addressed. E-mail: j.okeefe@ucl.ac.uk

circlelike on the right but were run in two series of interleaved and balanced order (fig. S1E). Seventeen of the 20 cells clearly remapped between the square and the circle: 12 remapped by changing rate (only firing in one or other shape), and 5 remapped their field position (firing in different places in the two shapes). The remaining three cells did not reach our criterion for remapping (*18*). Almost all of the cells abruptly switched from the squarelike to the circlelike pattern at the same transition point. This effect is quantified in Fig. 3A by comparing the similarity of each cell's firing in the octagons to that in the square and circle (*18*). A similar pattern was seen in the other three animals (Fig. 3, B to D left side).

The abrupt and coherent remapping of the place cell ensemble seems to require coordinated action, as in an autoassociative network, rather than to reflect cells independently responding to the same subtle environmental changes. For example, if each cell independently remapped at any of the five shape transitions, the probability of N cells remapping at the same point would be 0.2^{N-1} ($P < 10^{-11}$ for the 17 cells from rat 4 in Fig. 2; $P < 10^{-4}$ for rat 1 in fig. S2A; $P < 0.05$ for rat 2 and rat 3, Fig. 3E and fig. S2B, respectively). This impression is strengthened by the remapping pattern in two of the four animals. In one animal (rat 2), the cells remapped between the 2:6 and 3:5 octagons during the first series of probe trials, but remapped between the square and 1:7 octagons during the second series (Fig. 3, C and E). Significantly, all cells again switched at the same point. Another animal (rat 3) showed a similar pattern, remapping at the 1:7 to 2:6 transition in the second series (Fig. 3D), whereas the remaining two animals remapped at the same point in both series (Fig. 3, A and B).

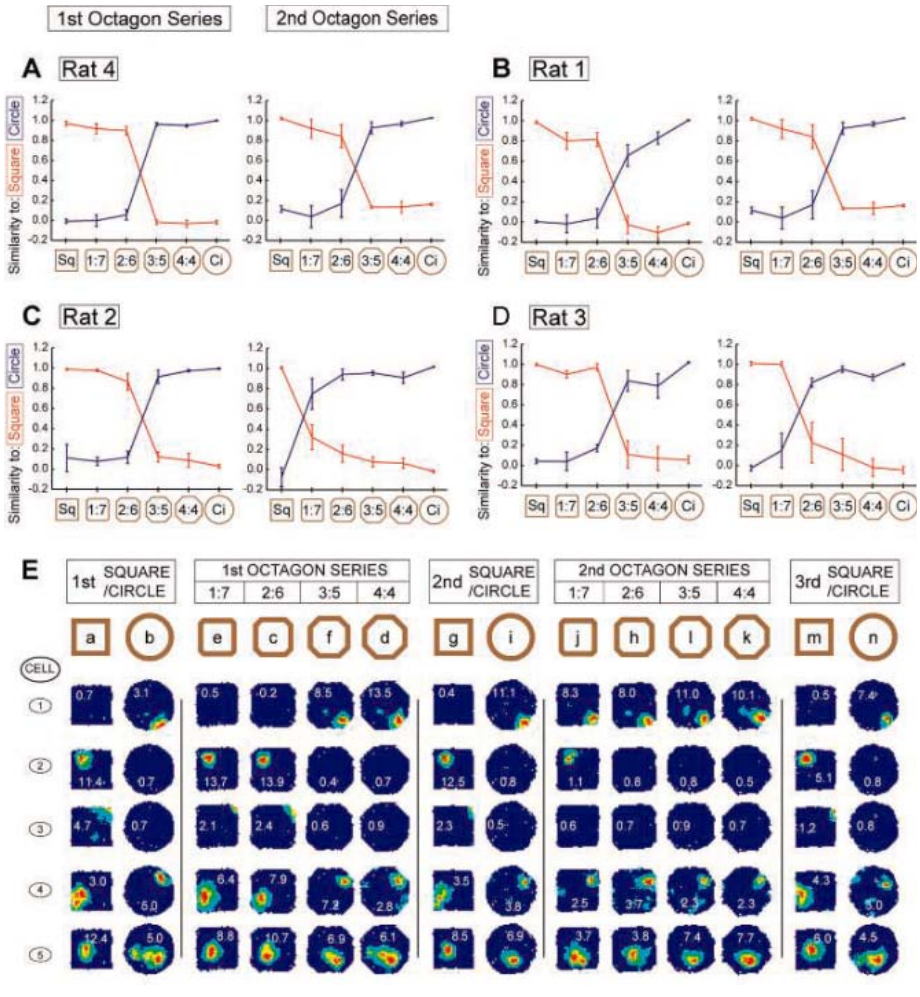

Fig. 3. Coordinated shift in square-to-circle switch point between the first and second octagon series. (**A** to **D**) Plots show the similarity of place cells' firing patterns in probe trials of varying shape to their firing patterns in square (red) or circle (blue) baseline trials [mean and SEM across cells (*18*)]. In the first series of octagons, all animals show abrupt remapping between the 2:6 and 3:5 octagons (A to D, left side); in the second series (right side), rats 4 (A) and 1 (B) again remap at this point, whereas rat 2 (C) remaps between the square and 1:7 octagon, and rat 3 (D) remaps between the 1:7 and 2:6 octagons. (**E**) Firing rate maps for all remapped cells for the two octagon series for rat 2.

Are attractor dynamics observable at the start of a trial? The firing patterns in intermediate shapes might take time to reach the circle or square representation, when starting from more intermediate representations (see fig. S3, A and B). The firing patterns in successive 10-s intervals from the start of each trial were examined (*18*) in our largest dataset (17 remapped cells, see Fig. 4, A and B). Several results should be noted. First, the similarity of the firing patterns in square and circle probe trials to square and circle baselines is stable across intervals (with the possible exception of the very first interval in the square). Second, the firing in the squarelike octagons (1:7 and 2:6) is already more squarelike than circlelike in the first 10-s interval, but slowly becomes more squarelike over the following 2 min. A similar pattern is seen in one of the two more circlelike octagons (4:4). This result indicates a surprisingly slow component to attractor dynamics that should be studied further with larger samples of cells (a similar trend that did not reach significance was seen for our next-largest dataset, the eight remapped cells in fig. S2A).

Previous experiments, including our own, did not find the integrated cooperative behavior among pyramidal cells shown here (*19*–*22*). For example, the place cell representation initially adjusts continuously to changes in environmental shape alone, consistent with purely feed-forward processing (*22*, *23*), and individual place cells slowly and independently learn to differentiate between square and circular environments made of the same material (*17*). One possibility is that synaptic modification in the CA3 recurrent collaterals is triggered by multimodal changes (e.g., of environmental shape, color, and texture) but not by unimodal changes, consistent with a hippocampal role in forming cross-modal associations between stimuli represented in disparate neocortical areas (*7*, *24*). Greater remapping was also seen when both proximal and distal cues were changed than when either set was changed alone (*25*).

The results suggest the operation of both pattern separation, which creates radically different representations from highly similar environmental inputs, and coordination of large numbers of place cells to create a global maplike representation of each environment (*2*, *4*, *5*, *7*, *10*, *26*). These functions are likely to originate in the hippocampus. Remapping has not been observed in its main cortical input, the entorhinal cortex (*27*, *28*), and we expect cells there would respond incrementally to the gradual changes in the octagon series. Although our recordings were made in CA1, following previous authors (*2*, *4*, *5*, *7*–*9*, *14*), we hypothesize that pattern separation takes place in the dentate gyrus, whereas autoassociative integration takes place in the CA3 recurrent collaterals (see fig. S3C). Four

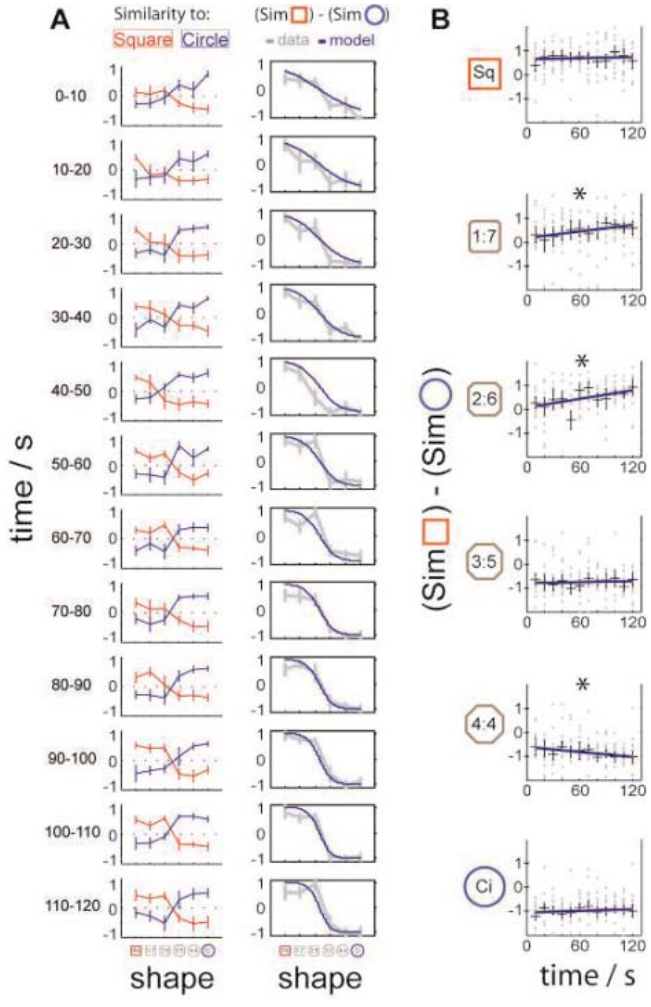

Fig. 4. Attractor dynamics of environmental representations. (**A**) Evolution of the firing pattern in successive 10-s intervals of trials in the different shaped environments. (Left column) The similarity of firing to that in square and circle baseline trials (mean and SEM for the 17 remapped cells in Fig. 2). (Right column) The difference in similarity of the firing pattern to square and circle baseline patterns. Gray line shows mean and SEM over cells of the difference between the red and blue curves shown in the left column. This becomes steadily more pronounced over time, and can be fitted by a sigmoid whose slope increases linearly with time (blue line: $y = 2/(1 + \exp[(a_0 t + a_1)(s - s_0)]) - 1$, where t is time in seconds, $s = 1$ to 6 corresponds to the series of shapes from square to circle, and $a_0 = 0.019$, $a_1 = 0.674$, $s_0 = 3.29$ were chosen to fit the data). (**B**) Firing patterns in intermediate 1:7 and 2:6 octagons become more squarelike over time, the patterns in 4:4 octagons become more circlelike, while the patterns in the square and circle remain unchanged (*$P < 0.05$ one-tailed, linear regression) (*18*).

examples are consistent with CA3's acting as an autoassociative network: the inability of mutant mice with disabled CA3 *N*-methyl-D-aspartate receptors to compensate for the removal of subsets of cues in the Morris water maze (*14*), the high sparsity of the CA3 representation (*20*, *25*), signs of hysteresis within it (*29*), and the coherent response of CA3 place cells to inconsistent rotation of two sets of cues. CA3 place cells mainly followed proximal cues, whereas CA1 cells followed combinations of proximal and distal cues (*21*).

Our finding of coherent activity of place cells specific to each environment has several potential functional consequences. Such representations or "charts" (*26*) could serve to reduce interference between environments by providing orthogonal representations for each. They would also allow the firing of large numbers of cells to be combined to provide an improved estimate of location (*30*). The capability for integrating information at distant locations with the representation of the current location may allow for short-cut and detour behavior (*10*, *31*). More generally, attractor dynamics are thought to underlie context-dependent recollection [as opposed to, for example, familiarity-based recognition (*32*)]. Thus, understanding the creation of new attractors, and their dynamics, may directly inform the nature and function of "context" in context-dependent episodic memory and its failure in amnesia. Finally, the ability to study this mechanism at the single unit level allows for electrophysiological, pharmacological, and genetic investigation of the mnemonic function of the hippocampus in health and disease.

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Supporting Online Material

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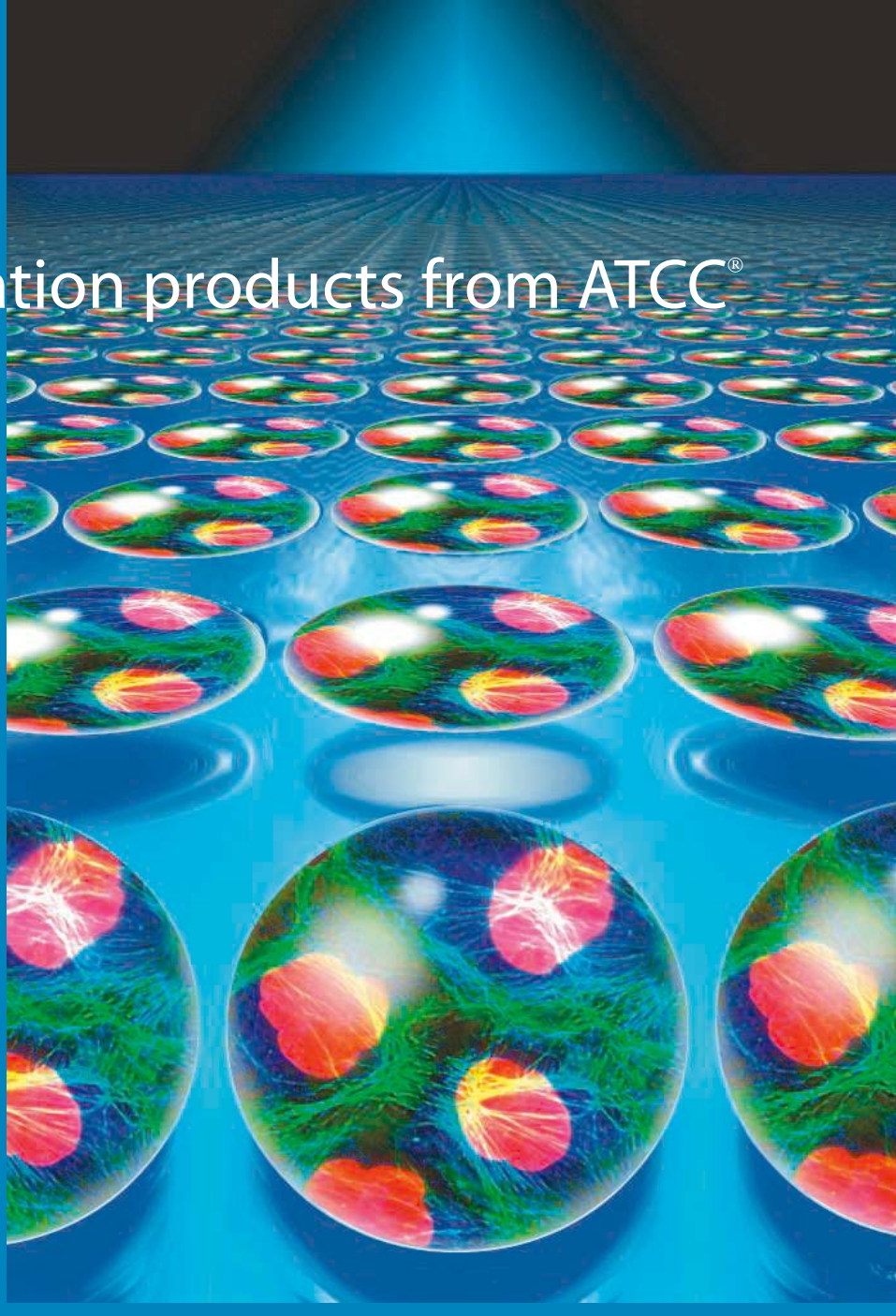

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A Chip of the Old Protein

Protein microarrays can provide more data faster and less expensively than traditional investigative methods. But only now have proteomic researchers begun to realize their value. BY PETER GWYNNE AND GARY HEEBNER

The term "proteome" refers to all of the proteins that a cell expresses. So identifying and measuring the quantities of proteins in a cell's proteome becomes a major goal of proteomic research. Carrying out those tasks at a specific time is difficult enough. But specialists in proteomics face the additional complication that the makeup of proteins in a given cell varies over time, depending on the cell's health and ambient conditions.

To help deal with those problems, researchers have begun to turn to protein microarrays. Otherwise known as protein biochips, the arrays have obvious value for research on proteins. Fundamentally, they provide a way to study proteomics in terms of what protein researchers actually need.

Arrays' Advantages

They also offer advantages over more traditional methods of proteomics research. "Protein arrays provide easier, more sensitive measurement," says Ray (Ruo-Pan) Huang, founder and interim president of **RayBiotech**. "Compared with 2-D gels and mass spectrometry, they allow you to detect protein expression in the proteome in an unbiased way." Dan Schroen, product manager for drug discovery products at **Nalge Nunc International** (NNI), offers a more expansive view of chips' promise. "Protein microarrays produce unsurpassed amounts of data in a shorter period of time, using less reagents and materials than a typical screen," he explains.

Despite those advantages, protein microarrays have so far gained only slow acceptance from the research community. "I don't feel that they have been fully appreciated yet," says Timothy Burland, president and CEO of **GWC Technologies**. "There are a lot of technical challenges, and a lot of people are not sure how to proceed." However, he adds, "Companies like GWC are helping researchers by providing specific suggestions on how to proceed."

Larry Gold, CEO and chief scientific officer of **SomaLogic**, argues that the industry bears some responsibility for that situation. "People want proteins chips; the demand is there," he explains. "But nobody has been able to deliver yet." Soleil Shams, founder and president of **BioDiscovery**, takes a similar view. "Certainly protein chips are not as mature as 2-D gels and other methods," he says. "But everyone is hopeful that we will get there."

Indeed, signs point to imminent expansion of the use of protein microarrays in and beyond the research laboratory. "They are used primarily in the academic area, but they are rapidly making inroads into the pharmaceutical area as well," says Santosh Arcot, product leader for array systems at **PerkinElmer Life and Analytical Sciences**. "Protein microarrays have begun to penetrate the clinical research area," adds William Rich, CEO of MORE ›››

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In this issue:

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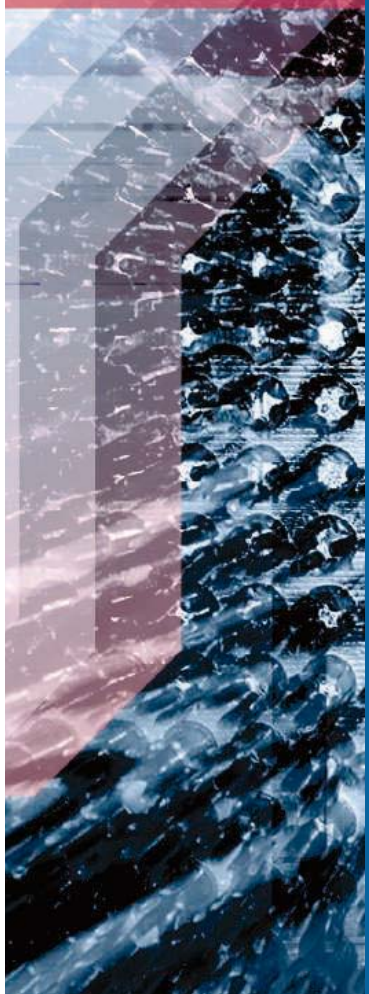

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Ciphergen Biosystems. "We've seen an expansion in publications to about 40 in the last three months – mostly in the clinical research area and most focused on biomarker discovery and translations into types of diagnostic assays."

Range of Uses

NNI's Schroen outlines the range of possible uses for protein microarrays. "For instance," he says, "protein-antibody interactions performed previously in ELISAs using the entire wells of a 96-well plate can now be miniaturized into multiplexed array features in the same wells or on a one-inch by three-inch slide. Microarray technology can also be employed to analyze protein-DNA interactions, which may have previously been assessed using time- and material-intensive approaches such as blotting or electrophoretic mobility shift assays. Clinical diagnostics represents perhaps one of the largest areas of potential impact and growth, as diagnostic products begin to enter the marketplace."

Whatever the application of their microarrays, users must choose between making their own and buying them off the shelf – although that decision is often made for them by the lack of appropriate off-the-shelf chips. "The decision has to do with the specific way they're performing their research and whether the chip exists commercially," explains Amy McCann, global product leader for array systems at PerkinElmer Life and Analytical Sciences. Commercial availability is a significant roadblock. "A key differentiator between genomics and proteomics is that you can buy a whole genome chip off the shelf," her colleague, Arcot, points out. "We're not there yet with protein chips."

Commercial protein microarrays that have reached the market have several advantages. "If your institute has the equipment and the facilities, you can maybe make your own," RayBiotech's Huang advises. "But if a commercial product is available it's probably better to buy it, as it takes a lot of time, effort, and money to make your own protein chip. There's also the issue of reliability and reproducibility."

To simplify in-house production of protein biochips, several companies have designed integrated systems specifically for producing protein microarrays. They include **BioIntegrated Solutions**, **Genetix**, **Genomic Solutions**, and PerkinElmer. "We're the tools provider for protein array fabrication, slides, scanning, and automated processing," McCann says. "Our Piezoarray is a noncontact printing system. We have a hydrogel-coated slide with a three-dimensional substrate that's superior for proteins as it's a protein-friendly environment. When the chips are ready to go, our ProteinArray Workstation processes them. And then we take the arrays to the ProScanArray whose analysis capability will quantitate your protein arrays and overcome the data bottlenecks."

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Issues of Attachment

Whatever type of molecules they use in their protein microarrays, researchers must ensure that the molecules attach to the arrays' surfaces. **BD Biosciences Clontech**, Genetix, **Greiner Bio-One**, and **Schleicher & Schuell** offer glass slides with treated surfaces that allow attachment of proteins. Treatment methods range from coating the glass surfaces with aldehydes to applying a matrix that creates a three-dimensional surface.

NNI offers several surface treatments, such as Maxisorb, aminosilane, aldehyde, epoxy, and lysine for low-volume microarrays. Each treatment offers different binding mechanisms that researchers can tailor to specific applications. The company also offers both glass and polymer microscope slides as well as treated ArrayCote multiwell plates and slides. Spotting a single well of such plates with multiple features enables scientists to multiplex their assays.

Greiner Bio-One, meanwhile, has recently introduced a low-cost arrayer that scientists can use to spot their glass slides manually. The benchtop device can put down up to 768 spots per slide. Each spot has a diameter of about 500 microns and uses between three and five nanoliters of protein material. The microarrayer contains two printers – one 8-pin and the other 32-pin – to match 96-well and 384-well plates.

Plexigen offers the geneCube, a three-dimensional array made from stacked geneCards, for assaying hundreds of proteins with up to a thousand samples. The system's stacked layers allow flow-through parallel processing under conditions determined by the substrate applied to each card or layer. It allows for flexible experimental design and a wide variety of applications.

Capture-Based Chips

Several companies, including BD Biosciences Clontech, RayBiotech, and **Zyomyx**, have developed high-volume capture-based chips. Designed to isolate proteins of interest from a sample, these protein microarrays detect specific cytokines, identify which standard proteins exist in a sample, and even determine whether a protein has been activated by phosphorylation. "We developed the first cytokine antibody array," recalls Rani Huang, RayBiotech's marketing director. "Our general cytokine array can detect up to 180 different proteins in one experiment. We also have angiogenesis arrays, inflammation arrays, chemokine arrays, and disease-related antibody arrays." Adds Ray Huang: "We offer low price, so that any lab is capable and equipped for routine use."

SomaLogic has decided to develop its protein microarrays using photoaptamers, which form specific covalent cross-links with target proteins when exposed to ultraviolet light. "Our business is spotting capture agents that catch proteins," Gold explains. "The cross-linking allows stringent washing, lower background, and enhanced specificity. In fact our good photoaptamers bind to and cross-link one protein only in serum – the target analyte."

The company focuses on clinical diagnostics, and particularly discovery of biomarkers and protein signatures. "However," says Todd Gander, senior director of corporate development and strategic MORE ›››

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planning, "we'll be available to clinical researchers through collaborations, and we will ultimately introduce research products, possibly through a strategic partner."

Scientists can also use protein microarrays to study interactions among proteins or peptides. Although relatively few companies have moved into this business, **Biacore** and **JPT Peptide Technologies** have released effective protein microarrays for interaction studies.

Ciphergen Biosystems has taken a very different approach to protein microarrays. Its technology avoids the use of antibodies or other ligands altogether. Instead the company's ProteinChip arrays use chromatographic surfaces, prepared by surface enhanced laser desorption/ionization technology, that enable the reproducible capture and study of unknown proteins from crude samples. "If you have different surfaces, such as anionic, cationic, and some kind of metal affinity, each one captures the proteins in a broad class," Rich explains. "If you adjust the pH, you can catch large subclasses. When you don't know what proteins you're looking for, you can run this technology broadly as a differential profiling method. And you can tune the selectivity to your taste. Of course, you can also directly create antibody, protein, and DNA capture versions of our chips using our reactive surface chemistry chips and your own bioaffinity molecules."

Labeling and Amplification

Detecting the contents of protein microarrays involves a variety of labeling and amplification chemistries, including fluorescent, luminescent, and radioactive methods. **Evrogen**, **Invitrogen** and **Sigma-Aldrich**, among other companies, provide tags for labeling proteins. And **Molecular Devices** (Axon), PerkinElmer, **Tecan**, and **TeleChem International** offer microarray scanners and imagers.

Labeling a protein can interfere with its function. Since most drug targets are proteins, a label-free method has obvious interest for drug discovery. GWC Technologies uses surface plasmon resonance (SPR) imaging to detect molecular interactions on protein biochips and other types of microarrays. The method detects the presence of a molecule on a gold surface by the change in the local index of refraction that occurs on adsorption. "It fits in as a label-free method that enables you to analyze proteins' interactions with other proteins, different ligands, and nucleic acids," Burland says. "Our SPRimager can analyze anything. It doesn't care about the chemistry, but the mass on the surface makes it very versatile. Even if your needs change, your analytical instrument need not."

After detection comes interpretation, which means bioinformatics. "The need for bioinformatics in particular and software in general in proteomics ranges from image analysis to managing all the data that are generated and performing quality control on the data," BioDiscovery's Shams says.

Companies such as **Accelrys**, BioDiscovery, and **MDL Information Systems** have developed suites of bioinformatic software to manage the storage and retrieval of data from protein microarray experiments, to mine those data sets, and to explore relationships among the data. BioDiscovery, meanwhile, has partnered with **Prolinx** to develop integrated software for analyzing protein microarrays. Why did they link up? "BioDiscovery is a leading software provider for gene expression and imaging analysis. Prolinx made protein chips," Shams explains. "It made a lot of sense to bring our experiences together."

Protein microarrays may have made slow progress toward acceptance by proteomics researchers until now, but continuing improvements make them increasingly compelling. "Protein chip surfaces, formats, materials, methods, and reagents are still emerging," NNI's Schroen says. "So it's an exciting time to be involved in this field."

Peter Gwynne (pgwynne767@aol.com) is a freelance science writer based on Cape Cod, Massachusetts, U.S.A. Gary Heebner (gheebner@cell-associates.com) is a marketing consultant with Cell Associates in St. Louis, Missouri, U.S.A.

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Ph.D., or preferably M.D./Ph.D., with at least four years of experience to run basic science laboratory. To design and perform clinical, in vivo, and in vitro studies in the field of cancer biology. Experienced in mammalian cell culture, isolation of DNA and RNA, PCR and RT-PCR, DNA constructs, preparation of cell lysates, immunomagnetic protein purification, Western blot, zymography, enzyme-linked immunosorbent assay, immunohistochemistry, and immunofluorescent staining, microscopy/confocal microscopy, and flow cytometry. Contact: **Dr. Richard L. Whelan, telephone: 212-342-1155; fax: 212-305-1981.**

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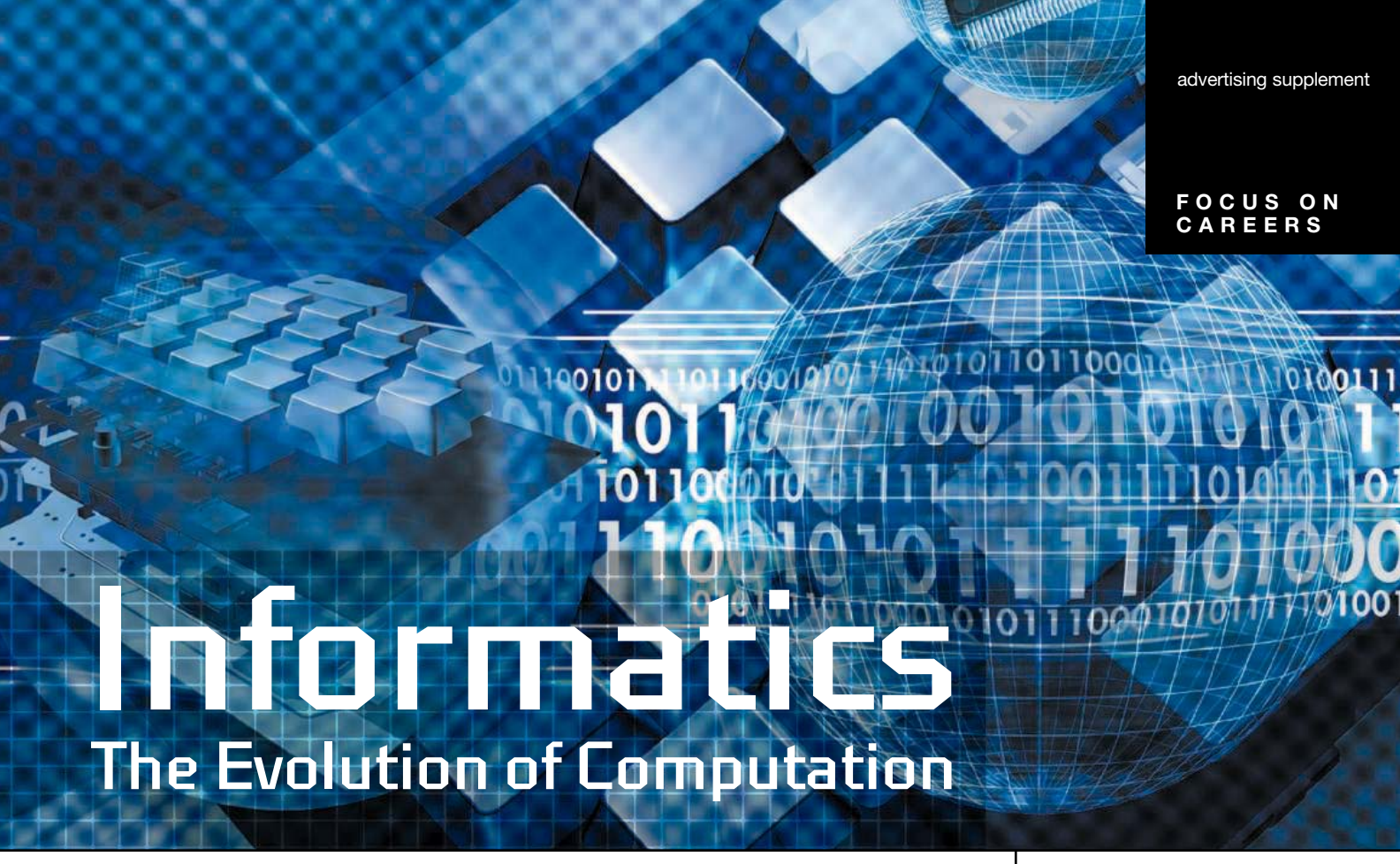

Informatics

The Evolution of Computation

FROM A TRADITIONAL PERSPECTIVE, INFORMATICS COMBINED COMPUTER SCIENCE AND MOLECULAR BIOLOGY. AS THIS FIELD GROWS, IT ATTRACTS EVER MORE AREAS OF EXPERTISE, INCLUDING ALL OF BIOLOGY, INDUSTRY, MATHEMATICS, STATISTICS, AND MORE. SOME OF THE EXPERTS INTERVIEWED HERE ADD THAT THIS FIELD IS NOT JUST HOT, BUT EXTREMELY HOT. **BY MIKE MAY**

Anyone in science today knows the word informatics. Even more, all modern life scientists know informatics as an exciting and dynamic field. Beyond people in this field, however, many scientists do not know the precise boundaries of informatics. In fact, it takes an expert to really keep up with the progress in this area and to know just how far the applications can go.

A wide variety of bioinformatics applications is under way at Invitrogen, a company that supplies products and services for research, drug discovery, and more. Claude Benchimol, senior vice president of research and development at Invitrogen, says, "Way back when, biology was an observational, analog science. With the sequencing of the human genome, biology was propelled into the digital age." That transformation, says Benchimol, required computers and mathematics.

Bringing computation and mathematics to biology kicked off the field of bioinformatics. According to Peter J. Munson, chief of the mathematical and statistical computing laboratory in the division of computational bioscience at the center for information technology of the National Institutes of Health: "Bioinformatics started as a cross-disciplinary field that combined classical molecular biology and computer science, but it was always broader." Today, Munson says that this field is almost too broad to limit its boundaries. He now defines bioinformatics as "a field that exploits computational tools in the service of life science." He adds, "It's like a big tent, and everyone is getting under it to some degree."

- **Accelrys**
  http://www.accelrys.com/
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  http://www.invitrogen.com/
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  http://www.nih.gov/
- **Virginia Polytechnic Institute and State University**
  http://www.vt.edu/

Calculating the Opportunities

For any field as large as informatics, the range of opinions about the field's potential covers some ground as well. According to the scientists interviewed here, bioinformatics is still growing. Bruno Sobral, executive and scientific director of the Virginia Bioinformatics Institute and professor of plant pathology, physiology, and weed science at Virginia Polytechnic Institute and State University, says, "I think bioinformatics is still pretty hot." He adds that this field experienced an initial rush and then some consolidation, but he still sees lots of positions. In fact, his institute had 40 open positions at the time of his interview.

Informatics

The Evolution of Computation


CLAUDE BENCHIMOL

Munson sees even more possibilities. He says, "Informatics is extremely hot in terms of job opportunities." He adds, "I would say job prospects are great for the right person."

In some industrial sectors, though, the bioinformatics positions may be growing more slowly. Darryl Gietzen, product manager for bioinformatics at Accelrys—a company that specializes in scientific software—says that informatics is a huge field, which experienced a great expansion over the past decade or so. "Since then," Gietzen says, "there has been a bit of a contraction. Now, we are seeing more moderate growth, not the booming advances from before."


DARRYL GIETZEN

Best Investments

The multidisciplinary nature of bioinformatics attracts scientists from a wide range of backgrounds. Remember, though, that bioinformatics focuses on biology. "If you want to be a card carrying bioinformatician," says Sobral, "you need to really learn biology—the language of biology, the semantics, the experimental design. It's not enough to just know what the data look like. You need to understand how experiments are conducted in the real world."

Mathematically minded scientists can find lots of opportunities in this field. Munson has a Ph.D. in mathematical statistics, which he considers a good fit. He says, "Informatics is the study of information, and statistics studies the quality of the information." He points out that an understanding of statistics can make science more efficient. "With good training in statistics," Munson says, "you avoid a lot of blind alleys that are easy to get trapped in, analogous to what happens when you are browsing the Internet." Nonetheless, Munson does not expect every bioinformatician to excel in statistics. Instead, he suggests knowing enough statistics to appreciate the value of the discipline and to put a statistician in your research group. "Bioinformatics is a team sport," he says. "If we can form interdisciplinary teams, it works much better."

In some cases, young scientists expect that they need to cover all of the bases alone. Gietzen says, "A lot of people expect strong statistical skills, a good computer coding background, and mix that with an understanding of biology, but I'd rather see someone with a strong scientific background." He does admit being biased, because he earned his Ph.D. in molecular biology. Still, he adds, "I find there is a shortage of people with informatic skills who really understand the science behind the informatics." In view of that shortage, Gietzen recommends that someone interested in this field consider a Doctorate in life science and then develop computing and statistical skills through additional course work or a postdoc.


PETER J. MUNSON

The Road Ahead

With a field as new as bioinformatics, young scientists should ask if this field can fuel an entire career. The experts interviewed here think that it can. In fact, Benchimol said that bioinformatics can absolutely carry a young scientist through a career. He says, "We won't see many major discoveries that do not include a bioinformatics component."

Already, bioinformaticians see new disciplines interested in information science. Munson says, "What seems to be happening is that new areas of biology are suddenly realizing that they need informatics so that they can grow." For example, Munson points out that scientists in transplantation medicine want to use informatics to find new markers for rejection of organs. He says, "It's a big field where surgeons have been the stars, but they need a whole team. We have been doing projects in this area that I wouldn't have thought of two years ago." In addition, informatics can be applied to critical care medicine, where physicians want to learn more about the causes of shock and organ failure. Munson says, "Critical care physicians are very keen on this, and there is a national symposium on functional genomics and critical care medicine, soon to meet for the third time here at NIH."


BRUNO SOBRAL

Future opportunities could also arise in fields that contribute to informatics. For example, fields like proteomics need new techniques to gather more data that can be fed into bioinformatic algorithms. Likewise, Sobral sees a need for better ways to handle data. He says, "It's unclear and debated whether the future of bioinformatic and current database technology are fully compatible. Relational databases have been there for some time, but will there be a new approach?" Beyond new high throughput tools and data management, Sobral adds, "We always want better algorithms."

In some cases, bioinformatics will change the very nature of not only science but the organizations that support it. That is already happening at Invitrogen. Benchimol says, "In our company, we are expanding the influence of bioinformatics. We have large initiatives in place to make bioinformatics one of the strong backbones by which we link science, people, and technology." In other words, informatics could eventually appear in all aspects of life science.

Mike May (mikemay1@verizon.net) is a communications consultant for science and technology based in Madison, Indiana, U.S.A.

explore Deep Science

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CAREERS IN INFORMATICS

CAREERS IN INFORMATICS

UTMB
The University of Texas Medical Branch

Faculty Position in Bioinformatics of Infectious Disease

The Department of Microbiology and Immunology at the University of Texas Medical Branch (UTMB) at Galveston is seeking an individual for a tenure-track faculty position interested in the application of bioinformatics to infectious disease. Rank, tenure status and salary will be commensurate with experience. The candidate is expected to establish an independent and externally funded research program and collaborate with other microbiologist, immunologist and infectious disease investigators. UTMB is designated as a Regional Center of Excellence (RCE) in Biodefense and Emerging Infectious Diseases and the site for one of two national biocontainment laboratories (Galveston National Laboratory). UTMB is exceptionally strong in infectious diseases research, and this position ideally offers an outstanding collaborative environment with scientists in the RCE, the Center for Biodefense and Emerging Infectious Diseases, as well as with established centers in vaccine development, structural biology, and proteomics, among many others. The position offers an attractive start-up package, as well as access to extensive computation resources at the University. UTMB is a member of the Gulf Coast Bioinformatics Consortia.

To apply, send a C.V. and names of four references to:

Chair
Bioinformatics Search Committee
Department of Microbiology & Immunology
The University of Texas Medical Branch
301 University Blvd.
Galveston, TX 77555-1075

UTMB is an Equal Opportunity/Affirmative Action University that proudly values diversity. Candidates of all backgrounds are encouraged to apply.

POSITIONS OPEN

St. Johns River Water Management District

Two positions for senior environmental scientists with expertise in aquatic systems management and restoration are available in north-central Florida. These positions entail advanced professional work based in Palatka, Florida managing team-based scientific assessments of water pollution in regionally significant lakes and rivers of the middle basin of the St. Johns River in central Florida near Orlando. These on-going pollutant analyses focus on three large lakes (Jesup, Monroe, Harney), segments of the St. Johns River, and two spring-fed rivers: Wekiva and Rock Springs.

Primary duties include long-term scientifically based management of water resources by providing scientific expertise in support of lake and river restoration in the Middle St. Johns River Basin; scientific data management, analysis, and reporting; Pollutant Load Reduction Goal (PLRG) development; providing assistance to the Florida Department of Environmental Protection in their development of Tads; and development of lake and river restoration alternatives. These positions manage contracts with consultants and university faculty to produce high quality work on schedule and within budget. Knowledge of limnology and lake management, river and stream ecology, water quality, and statistics. Skilled in data analysis, scientific writing, effective written and oral communication of scientific information; and constructive team participation. Ability to perform field work in lakes and rivers under adverse conditions.

- **Department:** Water Resources
- **Division:** Division Of Environmental Sciences
- **Location:** Palatka
- **Position Titles:**
  Environmental Scientist V: Min. $43,430.40 – Max. $75,566.40
  Environmental Scientist IV: Min. $46,051.20 – Max. $82,513.60
- **Apply by:** May 27, 2005
- **Qualifications include:** a bachelor's degree from an accredited college or university in biology, limnology, chemistry, botany, environmental science or related field and six years professional experience in the environmental field. A Ph.D. is preferred.

Competitive salary and attractive benefits. For additional employment information and to access our on-line application visit our web site at **SJRWMD.com**.

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Bethesda, Maryland 20892-0240
800.445.8283

NATIONAL INSTITUTE OF MENTAL HEALTH

The National Institute of Mental Health (NIMH), National Institutes of Health (NIH), Department of Health and Human Services (HHS), is seeking candidates for Chief, Experimental Therapeutics Branch, Chief, Psychotic Disorders Treatment Program, and Chief, Mood and Anxiety Disorders Treatment Development Program, within the Division of Adult Translational Research (DATR). The Experimental Therapeutics Branch plans, supports, and conducts programs of research, research training, and methods development to promote the clinical evaluation of novel pharmacological agents, devices, and interventions for mental illness and to evaluate new or off-label uses of approved or efficacious agents in the treatment of mental illness. In addition, the Branch aims to develop and support new research that integrates studies of treatment mechanism with efficacy studies as well as research establishing the utility of biomarkers to predict clinical treatment response. These positions will provide leadership and guidance within the area of experimental therapeutics across multiple grant and contract funding programs within the Branch, and will play important roles in establishing goals and directions of therapeutics initiatives and activities within the Branch and Division. The scientific and technical background appropriate for these positions could come from any of a large number of areas, including, but not limited to: psychiatry, neurology, human clinical trials, neuropharmacology, clinical pharmacology, clinical neuroscience, neuroimaging, psychology, genetics, etc. While not a requirement, regulatory and/or industry experience in CNS clinical development would be desirable. Candidates must have at least a Ph.D. Individuals filling positions will work closely with the Director, Division of Adult Translational Research. The ability to work both independently and collaboratively is required. Strong scientific, analytic, communication, and organizational skills are also required. Salary will be commensurate with experience. **Send CV and bibliography by email to Wayne S. Fenton, M.D. at wfenton@mail.nih.gov (Tel: 301-443-0081) by June 15, 2005.** With nationwide responsibility for improving the health and well being of all Americans, the Department of Health & Human Services oversees the biomedical research programs of the National Institutes of Health **(http://www.os.dhhs.gov).**

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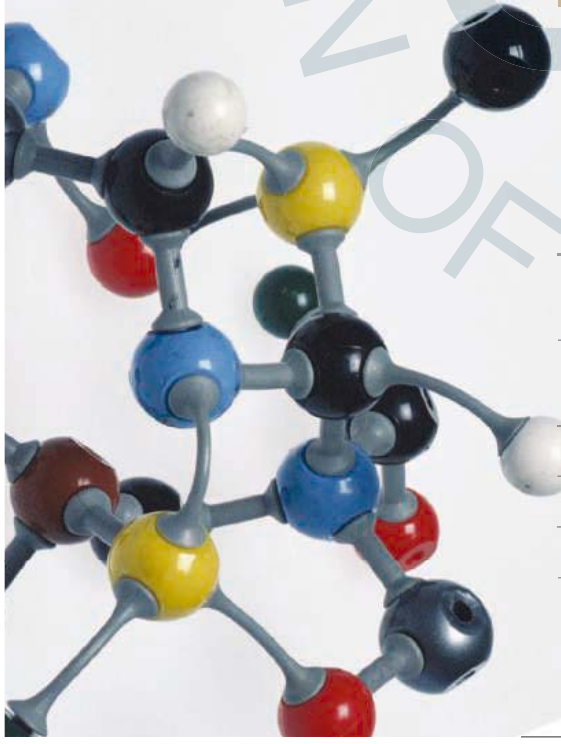

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FACULTY SEARCH
DEPARTMENT OF GENETICS
CASE WESTERN RESERVE UNIVERSITY
SCHOOL OF MEDICINE

Faculty positions at the assistant, associate, and full professor levels are available in the Department of Genetics for outstanding scientists involved in human genetics and genomics research. These investigators will join existing human genetics faculty whose research programs include the genetics and biology of human disease, complex traits and genomics, genetic epidemiology, and clinical genetics. Preference will be given to individuals working on cancer, metabolic diseases, or cardiovascular disease.

Twenty basic and clinical faculty base their research, teaching, and clinical activities in the Department of Genetics. Six recent faculty appointments have been in the fields of comparative genomics and complex traits, genetic epidemiology, developmental genetics, and clinical genetics. The Department has exceptional research and training programs in organisms ranging from Drosophila and C. elegans to mice and humans. State-of-the-art research laboratories with access to imaging, expression profiling, transgenic and genetic engineering, high throughput genotyping and sequencing, bioinformatics, and animal services significantly enhance research and training within the department.

Positions are available at the level of Assistant Professor (tenure-track), Associate Professor, and Full Professor, depending on experience and accomplishments. Preference will be given to applications who complement ongoing programs or who bring novel experience, expertise or research perspectives. Successful applicants are expected to develop an independent research program and to contribute to the Department training program. They will also have a M.D. or Ph.D., or both.

Applicants should submit a curriculum vitae, a brief statement of research and teaching interests, and arrange for at least three letters of reference. Applications and letters of reference should be submitted to:

Dr. Joe Nadeau
Faculty Search
Department of Genetics
Biomedical Research Building, 7th Floor
Case Western Reserve University
10900 Euclid Ave
Cleveland, OH 44106
Website: http://genetics.case.edu
Email: genetics_search@case.edu

Case Western Reserve University is an Equal Opportunity Employer encouraging excellence through diversity. Qualified women and minority candidates are encouraged to apply.

H·Y·M·S
THE HULL YORK MEDICAL SCHOOL

THE UNIVERSITY of York

Department of Biology

Research Positions in Immunology

Joint appointments of Hull York Medical School and the Department of Biology, University of York

Two postdoctoral scientists (refs: DR05151 and DR05152) and one graduate research assistant (ref: DR05153) are required to join an active research group studying immune regulation in a model of visceral leishmaniasis (Immunity. 2004 21:805; Eur J Immunol. 2005 35:498).

You will focus on defining cellular and molecular pathways of dendritic cell differentiation (DR05151) and on regulatory interactions between DC and CD4+ and CD8+ T cells (DR05152 and DR05153). All applicants should have a good background in cellular and/or molecular immunology and preferably experience of analyzing immune responses *in vivo* (required for post DR05152).

Informal enquiries can be made directly to Professor Paul Kaye, email: pmk2@york.ac.uk

Salary will be within the range £19,460 - £29,128 p.a. These MRC funded posts are available for a period of five years from 1 July 2005.

For further particulars and details of how to apply, please see our website: http://www.york.ac.uk/admin/persnl/jobs/ or write to the Personnel & Staff Development Office, University of York, Heslington, York YO10 5DD, quoting the appropriate reference number.

Closing date: 31 May 2005.

The University of York is committed to diversity and has policies and developmental programmes in place to promote equality of opportunity. It particularly welcomes applications from ethnic minority candidates.

The Hull York Medical School is an equal partnership between the Universities of Hull and York.

It operates in association with the NHS in North and East Yorkshire and Northern Lincolnshire.

www.hyms.ac.uk www.york.ac.uk

The University of Montana

Director and Tenure-track faculty positions in Biomolecular Structure and Dynamics

Applications are invited for a DIRECTOR and TENURE-TRACK faculty positions to strengthen research and graduate education in structure and dynamics of biological macromolecules. This recruitment is coupled to the development of a proposed Center for Biomolecular Structure and Dynamics, supported by a large NSF EPSCoR grant and an NSF ADVANCE grant (to support the hiring of women faculty in the sciences and mathematics). Successful candidates are expected to bring or develop a vibrant, externally funded research program in the area of structural biology, biophysics or structural biochemistry that may range from the study of single molecules to their assemblies in cells. Successful candidates will direct students, have demonstrated excellence in teaching and participate in graduate and undergraduate courses in their areas of expertise. Senior candidates will be considered for DIRECTORSHIP of the Center. The director must be conducting internationally recognized research. She or he must have the vision and qualifications for dynamic leadership, and a proven record of leadership qualities necessary to direct a world-class Center. It is expected that she or he will lead efforts to develop and obtain program-project and center grants, enhance graduate student recruiting and facilitate collaborations with existing departments. Successful candidates will become faculty members in the Division of Biological Sciences, **http://biology.dbs.umt.edu/dbs/**, the Department of Chemistry, **http://www.umt.edu/chemistry/**, the Department of Biomedical and Pharmaceutical Sciences, **http://www.spahs.umt.edu/pharmsci/**, or other suitable departments.

Applicants must have a doctorate and post-doctoral experience in chemistry, biochemistry, biology, biophysics or biomedical sciences. Demonstrated ability to attract NIH or NSF funding and/or contract research funding is essential. The search is ongoing and applications will be accepted immediately. Send letter of application, curriculum vitae, statement of current research plans, a statement of teaching philosophy and names, addresses, and telephone numbers of at least three references to: **Chair, BSD Search Committee, Division of Biological Sciences, University of Montana, Missoula, MT 59812-4824**. For more information go to the BSD website at **http://www.cas.umt.edu/biomolecular/** or contact **walter.hill@umontana.edu** or **sandy.ross@umontana.edu**.

The University of Montana is an EO/AA Employer and recipient of an NSF ADVANCE PACE award focused on the status of women in science. Applications from women, minorities, veterans, and persons with disabilities are encouraged. Positions are eligible for veterans' preference in accordance with State law.

INTERGENETICS INCORPORATED

InterGenetics is a cancer genetics-based company located in a state-of-the-art research park with over 500,000 sq. ft. of class A wet-lab and office space. The research park is located in the larger Health Sciences Center, a community recognized for its contributions in the medical industry. InterGenetics is seeking outstanding individuals for the following positions:

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InterGenetics has identified gene-gene and gene-environmental interactions that have been applied to the construction of predictive cancer risk models for breast cancer. Current analyses rely on computer intensive iterative resampling and data randomization to identify input variables. The candidate selected for this position will work with a team to provide insights into cancer risk model development methods and possibly take the lead on developing future models for other cancer risk tests.

Applicants must have a Ph.D. in Statistical Genetics or related discipline and 5+ years post-graduate work in the field of cancer risk and/or other complex disease modeling. An extensive genetics background and working knowledge of genetics is required.

MOLECULAR BIOLOGIST / RESEARCH LABORATORY MANAGER

The successful candidate will: participate in research and development to identify, improve and implement a genetics-based cancer risk model through to the commercial environment; contribute to the improvement of assay development and new platforms; write grants for additional research projects; writing manuscripts for peer reviewed publication; and contribute to new research in the development of follow-on tests and pharmacogenomic and targeted therapeutic research.

Applicants must have a Ph.D. in molecular biology with 5+ years research experience in genetic predisposition to disease, preferably cancer. The ideal candidate will have excellent writing skills and appropriate publications record for experience. This position will require creative and critical thinking and problem solving. Experience in high throughput PCR based assay development and validation required.

Compensation consistent with experience. Mail, e-mail, or fax resumes to: **InterGenetics Inc., Attn.: HR – SG/MB; 800 Research Parkway, Suite 390, Oklahoma City, OK, 73104. E-mail: boxt@intergenetics.com. Fax: 405-271-1725. Web site: www.intergenetics.com.**

EOE

DIRECTOR
W. M. KECK OBSERVATORY

The University of California and the California Institute of Technology formed the California Association for Research in Astronomy (CARA) to construct and operate the W.M. Keck Observatory. CARA provides oversight of the operation of the Keck observatory and its related equipment, instrumentation, support facilities and infrastructure through its Board of Directors.

The CARA Board seeks a Director for the Observatory to succeed Fred Chaffee, the present director, who is retiring. The Director reports to the CARA Board and is responsible for managing the operations of the observatory within budget so as to maximize its readiness and effectiveness for scientific research. The Director is responsible for recruiting and maintaining high quality technical and administrative staff, developing an annual budget for review and approval, and developing with the Science Steering Committee the short-range and long-range development plans for the observatory. The Director oversees the scheduling of the telescopes for science and engineering and acts as the primary interface with the astronomical user community. The Director maintains effective liaison with the CARA Board, the Science Steering Committee, the University of Hawaii, the local Hawaiian community and other external organizations, in each case ensuring that the Observatory is aware and responsive to their respective needs and desires. The Director is also responsible for maintaining a public outreach office and pursuing and managing public and private fund-raising activities, with guidance from the Board.

REQUIREMENTS: Ph.D. in astronomy or related field or equivalent relevant experience. Experience and demonstrated capabilities in managing a scientific research facility and working in a team-oriented environment are essential. The Director must also be skilled in written and oral communications and have the ability to work collaboratively among varied constituencies in order to achieve consensus.

SALARY RANGE: Dependent on background and experience and to be negotiated.

STARTING DATE: July 1, 2006

GENERAL INFORMATION: The Director will be resident at the W.M. Keck Headquarters in Waimea, Hawaii. The initial term of appointment will be from three to five years with the possibility of renewal. Further particulars of the appointment are available on request.

APPLICATIONS: Review of applications will be begun on **August 1, 2005**, and the recruitment will remain open until the position is filled. Applications together with the names of three references should be submitted in confidence to the following address:

Dr. Richard S. Ellis
Chair, Search Committee
Astronomy 105-24
Caltech
Pasadena, CA 91125

Email submission to **jlm@astro.caltech.edu** is also acceptable.

Computational Chemistry and Biology Opportunities at D. E. Shaw Research and Development

Extraordinarily gifted computational chemists, biologists, and other computational scientists are sought to join a rapidly growing New York-based research group that is pursuing an ambitious, long-term strategy aimed at fundamentally transforming the process of drug discovery.

Candidates should have world-class credentials in computational chemistry, biology, or physics, or in a relevant area of computer science or applied mathematics, and must have unusually strong research skills. Relevant areas of experience might include protein structure prediction, the computation of protein-ligand binding affinities, the study of biologically important systems using molecular dynamics and/or Monte Carlo simulation, and the application of statistical mechanics to biomolecular systems—but specific knowledge of any of these areas is less critical than exceptional intellectual ability and a demonstrated track record of achievement. Current areas of interest within the group include molecular dynamics simulation of functionally significant globular and membrane proteins, the prediction of protein structures and binding free energies, structure- and ligand-based drug design, characterization of protein-protein, protein-nucleic acid and protein-lipid interactions, and the development of algorithms for biomolecular simulations.

This research effort is being financed by the D. E. Shaw group, an investment and technology development firm with approximately $14 billion in aggregate capital. The project was initiated by the firm's founder, Dr. David E. Shaw, and operates under his direct scientific leadership.

We are eager to add both senior- and junior-level members to our world-class team, and are prepared to offer above-market compensation to candidates of truly exceptional ability. Please send your CV (including list of publications, thesis topic, and advisor, if applicable) to sciencemag-cc@desrad.deshaw.com.

D. E. Shaw Research and Development, L.L.C. does not discriminate in employment matters on the basis of race, color, religion, gender, national origin, age, military service eligibility, veteran status, sexual orientation, marital status, disability, or any other protected class.

D E Shaw & Co

The University of Chicago
The Division of Biological Sciences and the Pritzker School of Medicine

Herbert T. Abelson Chair

INSTITUTE FOR MOLECULAR PEDIATRIC SCIENCE

DEPARTMENT OF PEDIATRICS AND COMER CHILDREN'S HOSPITAL

The Department of Pediatrics and the Institute for Molecular Pediatric Science at the University of Chicago are seeking a candidate at the senior level to become the first Herbert T. Abelson Professor, a newly endowed chair position.

Candidates must have an outstanding record of achievement and innovation in a research field related to hematopoiesis, malignant or nonmalignant hematologic disorders, or hematopoietic transplantation. The successful applicant is expected to be a recognized leader in the field with a commitment to collaboration, teaching of medical students and residents, and mentoring of junior faculty. The successful candidate will be expected to conduct an extramurally funded clinical or laboratory based research program, and to participate fully in the academic mission of the institution.

Candidates with clinical experience in pediatric hematopoietic stem cell transplantation will have the option to direct the clinical program in the newly opened state of the art Comer Children's Hospital and a leadership role in the Institute is also an option.

The position is associated with a substantial recruitment package including significant funding and new laboratory space.

The clinical and biomedical environment at the University of Chicago is outstanding for this position. An impressive scientific faculty currently exists within many interactive groups including the Committee on Immunology, the Cancer Research Center, the Ben-May Cancer Research Institute and the Howard Hughes Medical Institute. The environment will be enhanced further with the growth of the Institute for Molecular Pediatric Science, which will be housed within a newly established, multi-disciplinary research institute with world class facilities.

Interested individuals should contact:

Albert Bendelac, M.D., Ph.D.
Professor of Pathology
Chairman, Committee on Immunology
The University of Chicago
5841 S. Maryland Ave., M/C 1089
Chicago, IL 60637
773-834-8646
abendela@bsd.uchicago.edu

Please include your curriculum vitae and a statement of your research accomplishments and future interests.

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SCOTT & WHITE

College of Medicine
The Texas A&M University System Health Science Center

Pediatric Hematology-Oncologist

The Section of Pediatric Hematology/Oncology at **Scott and White Clinic** and the **Texas A&M University System Health Science Center College of Medicine** (TAMUS HSC-COM) are seeking a clinician scientist with current research grants for a faculty position in a rapidly growing program. The candidate should be BE/BC in pediatric oncology and committed to an academic career. The successful candidates will join and enhance ongoing efforts in basic and translational research, with an institutional commitment to building a world-class experimental therapeutics program. An outstanding start-up package includes high quality laboratory space, excellent benefits and competitive salaries commensurate with academic qualifications. The position guarantees 75% protected time for research activities.

Scott & White Clinic is a 500+ physician directed multi-specialty group practice that is the leading provider of cancer care in Central Texas. Scott and White Clinic and the 486 bed tertiary Scott & White Memorial Hospital is the main clinical teaching facility for TAMUS HSC-COM. Outstanding clinical practice and laboratory facilities on campus that perform state of the art molecular and cellular biology research, flow cytometry, genomics and biostatistics are in place to support the research effort.

Please contact: **Don Wilson, M.D. Professor and Chairman, Department of Pediatrics, Scott & White, 2401 S. 31st, Temple, TX 76508. (800)725-3627 dwilson@swmail.sw.org Fax (254) 724-4974.**

For more information about Scott & White, please visit www.sw.org For Texas A&M www.tamhsc.edu. Scott & White is an equal opportunity employer.

CHAIR
DEPARTMENT OF MEDICINE
UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE

The University of Pittsburgh School of Medicine and the University of Pittsburgh Medical Center (UPMC) are seeking a chair for the Department of Medicine. The department comprises nearly 400 faculty members in 11 divisions. Basic, translational, and clinical research in the department have experienced rapid growth; and the divisions emphasize collaborative research (program project and center grants) as well as individual grant support. Department faculty provide more than one-third of the teaching to medical students and postgraduate training at the residency and fellowship levels.

The University of Pittsburgh School of Medicine is enjoying unparalleled growth in its clinical, research, and academic missions. The University of Pittsburgh is currently ranked 7th among educational and research institutions in NIH funding. The Department of Medicine alone has $72 million in sponsored research support. UPMC is one of the nation's largest academic health care systems, encompassing more than 35,000 employees; more than 5,000 physicians; and 18 tertiary care, specialty, and community hospitals serving 29 counties throughout western Pennsylvania.

Candidates should have major experience in academic medicine, including demonstrated leadership in directing clinical, research, and training programs, and should qualify for the academic rank of professor with tenure.

The University of Pittsburgh and UPMC are affirmative action, equal opportunity employers.

Please send curriculum vitae and bibliography to the chair of the search committee:

Arthur S. Levine, M.D.
Senior Vice Chancellor for the Health Sciences and Dean, School of Medicine
University of Pittsburgh
Scaife Hall, Suite 401
3550 Terrace Street
Pittsburgh, PA 15261

Phone: 412-648-8975
Fax: 412-648-1236
e-mail: alevine@hs.pitt.edu

University of Pittsburgh
School of Medicine

Directors of Research

The Italian Institute of Technology ("IIT"; **www.iit.it**) is a Foundation created to promote scientific research and technological innovation at the highest levels in Italy. Established by the Ministry of Economy and Finance and the Ministry of Education, University and Research with a consistent funding, IIT aims at becoming an international centre of excellence for scientific research and training in high technology, with active participation of private organizations. During its start-up phase IIT intends to set up state-of-the-art research programs in Nano-biotechnologies, Neuroscience and Robotics. The IIT scientific plan and the background material, approved by the Steering and Regulatory Committee, provide more detailed information and are available for download on IIT website.

In order to start scientific research activity, IIT invites applications for the following positions:

Directors of Research in Nano-biotechnologies

Directors of Research in Neuroscience

Directors of Research in Robotics

Successful candidates will start IIT research activities in the respective fields, developing a detailed research program, organizing and leading a research team and building laboratories in IIT definitive site in Genoa (I). Adequate laboratory space, start-up budget and equipment will be provided. Directors of Research will also develop close collaboration with industry and other research institutions.

Applicants should have an established record of significant scientific accomplishments, as well as scientific leadership and administrative skills. To ensure full consideration, **candidates will have to submit applications (electronic format preferred), with detailed cv, by the end of May** to:

Simone Collobiano
applications@iit.it
Fondazione IIT
Via Sicilia, 194
00187 – Rome, Italy
ph: +39 06 4201 0848

Short-listed candidates will be invited for a talk, which will take place in Genoa (I) during the first 2 weeks of July. On this occasion candidates will have the opportunity to present their current research activities and scientific goals.

Italian Institute of Technology is an Equal Opportunity Employer

Professorship (W2) in Nanobiotechnology / Biophysics

Fachhochschule Lausitz (FHL) is undergoing substantial growth and expansion in biotechnology. Within the life science branch the FHL is developing a consecutive Bachelor/Master course with main foci on Applied Microbiology and Tissue Engineering. To support the molecular approaches scientists experienced in the handling of single molecules are invited to apply for a professorship in Nanobiotechnology. The ideal candidate should have a strong background in biophysics and will establish his/her own research group. The new professor will teach biophysics in bachelor courses and is expected to participate in an educational program for international master students. Fields of interest might be nanoparticle based diagnostics, imaging of cellular processes, bioselective surfaces, molecular templates/ semisynthetic conjugates, organo electronics or sparse cell isolation. Cooperation with the colleagues of the department and application for grants is expected.

The FHL is interested in increasing the number of female employees. Thus, qualified female scientists are explicitly encouraged to apply for the position. Hiring priority is given to severely handicapped applicants with equal qualifications. The position is initially available for a period of 5 years with the option for tenure. Please contact christian.schroeder@FH-LAUSITZ.de for further information. Applications with the usual information should be sent to:

Fachhochschule Lausitz, Präsidentin, Postfach 10 15 48, 01958 Senftenberg, Germany

Application deadline: 6th June 2005

University of Zurich

The University of Zurich has implemented a Research Priority Program on the

"Foundations of Human Social Behavior"

Researchers in this program examine the determinants of pro- and antisocial human behavior from the perspective of economics, psychology, neurobiology, and philosophy. Research in the Priority Program involves, *among other things*, the analysis of the neural and emotional bases as well as the social and economic implications of empathy, fairness, reciprocity, equity, honesty, deception, and norm violations. In the context of this research endeavor, the Faculty of Economics, Business Administration and Information Technology of the University of Zurich seeks applications for

Assistant Professorships

in the fields of

Neuroeconomics, Social Cognitive Neuroscience, Behavioral Economics

Positions are available starting in January 2006. Applicants should have a high research potential and should be interested in examining the foundations of human social behavior with economic, psychological, or neuroscientific methods.

The professorships will be embedded in a strong research group at the Institute for Empirical Research in Economics (**http://www.iew.unizh.ch/home/fehr/**) with good access to neuroimaging techniques and other neuroscientific tools as well as the excellent facilities at the behavioral economics laboratory at this Institute. For further information on the available positions please contact Prof. Dr. Ernst Fehr (**efehr@iew.unizh.ch**).

Applicants should submit their application before June 10, 2005 to: Prof. Dr. Wehrli , Dean of the Faculty of Economics, Business Administration and Information Technology of the University of Zurich, Rämistrasse 71, CH-8006 Zurich, Switzerland.

Interested candidates should send their curriculum vitae, recent publications and research papers, and two letters of recommendation.

Leibniz Gemeinschaft

Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie - Hans-Knöll-Institut -

HKI

The Leibniz Institute for Natural Products Research and Infection Biology identifies natural products and characterizes the infection process of pathogenic fungi.

The Institute is expanding its research activities in the field of infection biology and is recruiting a

Junior Research group in Cellular Immunobiology

We are searching for an excellent, highly motivated researcher, who is interested in the response of monocytes and macrophages towards human pathogenic fungi and/or who works with animal models. The research group will include the position of the head and two coworkers and has an attractive budget. The junior group will be associated and interacting with the Department of Infection Biology (head Prof. Dr. Peter F. Zipfel) and is particularly suited for a Postdoctoral fellow abroad, who is planning to return to Germany.

For further information see **www.hki-jena.de**

UNIVERSITY OF OXFORD

Mathematical and Physical Sciences Division

Department of Physics

in association with Christ Church

University Lecturership in Experimental Quantum Optics

The Department of Physics proposes to appoint a person with a proven record of research to The Hewlett-Packard Lecturership in the sub-department of Atomic and Laser Physics. The post is a University Lecturership that will be held in conjunction with a Studentship at Christ Church, and will begin on 1st October 2005 or as soon as possible thereafter.

Preference will be given to experimentalists with recognised expertise in quantum optics and in light-matter interactions in the quantum regime, including applications in quantum technology, such as information processing. He or she will be expected to lead an internationally excellent research effort and be an effective teacher at both the undergraduate and postgraduate levels. It is expected that the successful candidate will interact closely with existing experimental programmes in the Department and, if appropriate, in the UK Quantum Information Processing Research Collaboration, in which the Department participates (See http://www.physics.ox.ac.uk/al/index.htm for a description of current research in the Department.)

The combined University and College salary will be according to age on a scale that currently peaks at £45,707 p.a., in addition to which the College pays a number of allowances as described in the Further Particulars. Further Particulars are available on the departmental website or from Professor Ian Walmsley, Department of Physics, Clarendon Laboratory, Parks Road, Oxford OX1 3PU, Tel: (01865) 272205, Fax: (01865) 272375, i.walmsley1@physics.ox.ac.uk The application procedure is described therein. Applications, quoting reference number DU05/013, should be submitted by 1st June 2005.

The University of Oxford and Christ Church are Equal Opportunities Employers.

ENDOWED PROFESSORSHIP IN INTEGRATIVE BIOLOGY

The Department of Biology is launching an initiative in Integrative Biology to meet the challenge of developing new interdisciplinary research and teaching at the interface of biology with physics, mathematics, chemistry, computer science and/or engineering. As part of this effort, we are seeking to fill a newly established endowed professorship, made possible by a gift from the Lucille P. Markey Charitable Trust. We seek a distinguished scientist whose research interests cross one or more of those interfaces. This endowed professorship will be supported with a generous allotment of space and start-up funds. The tenured Markey Professor will establish a vigorous research program and provide leadership in the future growth of interdisciplinary research and training within our department. Individuals whose research would build bridges to the physical or quantitative sciences, while strengthening existing programs within our department, will be favored. Areas of interest include (but are not restricted to) systems biology, epigenetics, biological chemistry, environmental sciences, quantitative genetics, genomics, and imaging at the cellular level. This professorship will contribute to strengthening the Division of Biology and Biomedical Sciences, a joint program providing important intellectual links between the departments in the School of Medicine and in the faculty of Arts and Sciences.

Consideration of applicants will begin on **June 6, 2005** and continue until the position is filled. Letters of application, accompanied by curriculum vitae, brief statements of research and teaching interests, reprints of up to three selected papers, and the names and affiliations of three persons who can be contacted for letters of recommendation should be sent to:

Markey Professor Search
Washington University
Department of Biology
Campus Box 1137
One Brookings Drive
St. Louis, MO 63130-4899

Women and members of minority groups are encouraged to apply.
Washington University is an Affirmative Action Employer.

DIRECTOR

LIFE SCIENCES INSTITUTE UNIVERSITY OF BRITISH COLUMBIA

Applications are invited for the position of the Scientific Director of the Life Sciences Institute (LSI). We seek a senior scientist with an established, international reputation in basic biomedical research and a strong record of scientific leadership.

The Life Sciences Institute will house up to 90 investigators from four Faculties who study molecular, cellular and physiological sciences as members of research groups. The LSI is housed in the Life Sciences Centre, a new state of the art facility that will also house the Centre for Disease Modelling, and a large Biocontainment Level 3 facility (see www.lsi.ubc.ca).

The Director will develop and lead the research priorities of the LSI, will encourage interdisciplinary research, and will identify and actively pursue opportunities for fund raising. The Director will report to the Deans of Medicine and Science and ultimately, the Provost. The successful applicant should be eligible for appointment as a tenured professor within an appropriate department if he/she is not currently a UBC faculty member. Salary will be commensurate with qualifications and experience. The successful applicant may be eligible to apply for a CRC Tier I chair.

Qualified applicants are invited to submit a concise statement of their vision for the LSI, a curriculum vitae, the names of three references, and a summary of their current research program to: **Dean of Science, University of British Columbia, 1505-6270 University Blvd., Vancouver, BC, V6T 1Z4.**

UBC

The deadline for submission of applications is **May 31, 2005**, and the anticipated starting date is Sept. 1, 2005, or by mutual agreement. This position is subject to final budgetary approval.

UBC hires on the basis of merit and is committed to employment equity. We encourage all qualified persons to apply; however, Canadians and permanent residents of Canada will be given priority.

HEAD/CHAIR, DEPARTMENT OF PHARMACAL SCIENCES
Harrison School of Pharmacy
Auburn University

Auburn University's Harrison School of Pharmacy is inviting applications and nominations for the position of Head/Chair, Department of Pharmacal Sciences. This is a tenure-track position at the **ASSOCIATE PROFESSOR** or **PROFESSOR** level.

Applicants must possess a Ph.D. degree in pharmaceutical sciences or a related field, and possess academic credentials sufficient to meet tenure eligibility requirements of Auburn University. The ideal candidate should have an outstanding scientific background in the area of drug-related sciences, an established and ongoing record of obtaining extramural funds, editorial board or study section experience, and the proven abilities to foster an interdisciplinary approach to research in order to assist the School toward achieving the next level of biomedical research. Applicants should possess good communication and interpersonal skills, demonstrated experience in leadership and management to ensure success in the operations of an academic department and school, and the ability to recruit and mentor new faculty. Salary will be competitive and commensurate with education and experience. Candidates selected for this position *must be able to meet eligibility requirements for work in the United States, and must be able to communicate in English.* Review of applications will begin May 15, 2005, and continue until the position is filled. A full position announcement is available at the School's **website: http://pharmacy.auburn.edu/professionals/positions.htm.**

Women and ethnic minorities are encouraged to apply. Auburn University is an Equal Opportunity/Affirmative Action Employer.

FACULTY POSITION THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

The Department of Anatomy at the University of Mississippi Medical Center invites applications for a tenure-track faculty position at the rank of **ASSISTANT PROFESSOR** or **ASSOCIATE PROFESSOR.** Preference will be given to candidates with prior/proven teaching experience. Teaching will be in medical and in dental gross anatomy. Opportunity exists for collaborative research; scholarly efforts in anatomical education, as broadly defined, will be especially encouraged. Applicants should send curriculum vitae and supporting documents to: **Chair, Department of Anatomy, The University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216-4505. E-mail: dhaines@anatomy.umsmed.edu.** Applicants must arrange to have three letters of recommendation sent to the above address. Review of applications will begin immediately. *Equal Opportunity Employer, Minorities/Females/Persons with Disabilities/Veterans.*

BIOINFORMATICS ANALYST

ExonHit Therapeutics, a cutting-edge company in the field of genomics, is seeking a bioinformatics and information technology specialist. Responsibilities: develop and expand the products for our SpliceArray platform using proprietary computational tools; maintenance and development of internal bioinformatics platform; and managing information technology architecture.

Requirements: one to three years of experience in biological sequence information analysis focusing on sequence homology and genomic mapping, and Solaris or Linux. Knowledge in relational database management, Java, and XML is desirable along with the ability to work in a team-oriented atmosphere. B.S./M.S./Ph.D. Salary commensurate with experience.

ExonHit Therapeutics offers a highly competitive package and a challenging atmosphere. Please e-mail your curriculum vitae to: **jobs@exonhit-usa.com,** referencing job code B-3. Visit **website: www.exonhit.com.** *Equal Opportunity Employer.*

POSITIONS OPEN

NATIONAL SCIENCE FOUNDATION
Division of Environmental Biology

The National Science Foundation's Division of Environmental Biology (DEB) is seeking qualified candidates for current and future positions of **PROGRAM DIRECTOR** in the following areas of expertise: ecological biology, ecosystem science, population and evolutionary processes, systematic biology, and biodiversity inventories. Program Directors are responsible for program planning and administration and for furthering the goals of the NSF and DEB. More information about DEB can be found on its **website: http://www.nsf.gov/div/index.jsp?div=DEB.** Positions may be filled as a one- or two-year visiting scientist (VSEE) or a federal temporary appointment with a salary range of $88,369 to $137,713, depending on qualifications and experience. Alternatively, this position may be filled under the terms of the Intergovernmental Personnel Act (IPA). Additional information on the VSEE and IPA program can be obtained at **website: http://www.nsf.gov/about/career_opps/.**

Applicants must possess a Ph.D. in biology or in an equivalent discipline, plus six or more years of successful research, research administration, or managerial experience beyond the Ph.D. Familiarity with NSF policies and practices, administrative experience, and recognized stature among peers are desirable. Interested individuals should submit curriculum vitae via e-mail and contact: **Dr. Michael Willig, Division Director (telephone: 703-292-8480; e-mail: mwillig@nsf.gov)** or **Dr. Penelope Firth, Deputy Division Director (telephone: 703-292-8480; e-mail: pfirth@nsf.gov)** for further information about the positions and the application process.

NSF is an Equal Opportunity Employer committed to employing highly qualified staff that reflect the diversity of our nation.

PROGRAM MANAGER FOR HYPOXIA RESEARCH

The National Oceanic and Atmospheric Administration's Center for Sponsored Coastal Ocean Research (CSCOR), National Centers for Coastal Ocean Science (NCCOS), National Ocean Service (NOS), is seeking a highly qualified candidate to lead a nationwide program of extramural research on hypoxia and eutrophication, one of the nation's leading coastal management problems. This research is required for federal, state, and local agencies to understand and predict the causes and impacts of hypoxia and nutrient pollution in support of informed ecosystem-based management decisions. This position will both oversee the prioritization of research topics and the conduct of research funded to address these topics. This position is for **OCEANOGRAPHER**, GS-1360-13/14, is located at the NOAA offices in Silver Spring, Maryland, and will require some travel.

For further information about the position, please visit **website: http://www.cop.noaa.gov** or contact **e-mail: coastalocean@noaa.gov.**

The U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), U.S. Vegetable Laboratory, Charleston, South Carolina, is seeking a permanent full-time **SCIENTIST** to research and develop control methods for diseases of watermelon, especially the emerging disease known as "vine decline." Research assignment will include: the etiology of diseases, development of pathogen identification procedures, characterization of the pathogenic and genetic variability in disease-causing organisms, identification of resistances to diseases in watermelon, and the characterization of the nature, durability, specificity, and genetics of identified resistances to facilitate their incorporation into improved watermelon germplasm. Salary range of $50,541 to $93,643. For details and application directions, see **website: http://www.afm.ars.usda.gov/divisions/hrd/.** To have a printed copy mailed, **telephone: 843-402-5300.** *U.S. citizenship is required.* Announcement closes June 8, 2005. Visit the ARS **website: http://www.ars.usda.gov.** *USDA/ARS is an Equal Opportunity Employer and Provider.*

POSITIONS OPEN

PHARMACOLOGIST (CHAIR)

The American University of the Caribbean (AUC), a 25-plus-year-old accredited medical school with over 3,000 graduate physicians, is pleased to announce an opening for the Chair of Pharmacology, Ph.D. and/or M.D. Rank is commensurate with experience.

We seek an individual with experience and expertise, who both enjoys and is dedicated to teaching. The course in medical pharmacology will be team taught. Individuals familiar with U.S. medical education and evaluation systems are encouraged to apply. All lectures are in English, with PowerPoint formats preferred. The majority of the basic science AUC faculty being drawn from North America and the European Union, with clinical clerkships in the United States, United Kingdom, and Ireland.

AUC (**website: http://www.aucmed.edu**) is in a new, up-to-date facility on the delightful island of St. Maarten in the Netherlands Antilles, some three hours by air from Miami, Florida.

Interested parties should send their curriculum vitae and the names of three references with coordinates to: **B. Salafsky, Ph.D.,** Dean, Basic Sciences, **e-mail: buzs@aucmed.edu.**

PHYSIOLOGIST

The American University of the Caribbean (AUC), a 25-plus-year-old accredited medical school with over 3,000 graduate physicians, is pleased to announce an opening for an M.D. and/or Ph.D. physiologist. Rank is commensurate with experience.

We seek an individual with experience and expertise, who both enjoys and is dedicated to teaching. In particular we seek a candidate with a strong background in renal and pulmonary physiology, who can join in team teaching medical physiology. Individuals familiar with U.S. medical education and evaluation systems are encouraged to apply. The majority of the AUC faculty are drawn from North America and the European Union, with clinical clerkships in the United States, United Kingdom, and Ireland.

AUC (**website: http://www.aucmed.edu**) is in a new, up-to-date facility on the delightful island of St. Maarten in the Netherlands, Antilles, some three hours by air from Miami, Florida.

Interested parties should send their curriculum vitae and the names of three references to: **Dr. Steve Blevins,** Chairman, Department Physiology, **e-mail: sblevins@aucmed.edu**.

RESEARCH ASSOCIATE POSITIONS
Case School of Medicine

Two Research Associate positions are immediately available in the Department of Physiology and Biophysics at Case School of Medicine. Candidates should have a Ph.D or an equivalent degree and have experience either in patch clamp technique or calcium imaging and/or whole animal physiology in rats and mice. Salaries are highly competitive and commensurate with experience. Interested applicants should electronically submit curriculum vitae along with a cover letter and three references on or before June 15, 2005, to: **Ms. Marianne Sperk,** Department of Physiology and Biophysics (**e-mail: mxs45@case.edu**). *In employment, as in education, Case Western Reserve University is committed to Equal Opportunity and world class diversity.*

POSTDOCTORAL ASSOCIATE

Position for a Molecular Biologist with a Ph.D. and/or M.D. degree to analyze events leading to malignant cutaneous melanoma in our melanoma-susceptible transgenic mice. This information is expected to provide a basis for new experimental treatments of the disease. Please send your curriculum vitae, including a summary of predoctoral work; a list of your publications, briefly stating your laboratory role in each multiauthored paper; and names of three references to: **Dr. Beatrice Mintz,** Fox Chase Cancer Center, Philadelphia, Pennsylvania, either by **e-mail: beatrice.mintz@fccc.edu, or by fax: 215-728-3574.** *Equal Opportunity Employer.*

Department of Health and Human Services
National Institutes of Health
Office of the Director
Officer of Extramural Research (OER)

The National Institutes of Health (NIH) in Bethesda, Maryland, the world's largest medical research facility, seeks applications from exceptional candidates for the challenging position of **Director, Office of Laboratory Animal Welfare**, located in the Office of Extramural Research. The NIH, a component of the Department of Health and Human Services, is the principal health research agency of the Federal Government. The NIH Extramural Program is the largest single source of funding for biomedical and behavioral research in the United States. Extramural research represents approximately 85% of the NIH budget.

The Director, Office of Laboratory Animal Welfare, provides executive leadership and direction to the Office of Laboratory Animal Welfare which is responsible for developing and coordinating appropriate Public Health Service (PHS) regulations, policies, and procedures on the humane care and use of laboratory animals involved in research conducted or supported by any component of the PHS. The Director, Office of Laboratory Animal Welfare, reports directly to the NIH Deputy Director for Extramural Research.

For full information concerning the duties and responsibilities of this position, salary and benefits available, required qualifications, and mandatory application procedures, interested candidates should visit the OER website at:
http://grants.nih.gov/grants/oer_vacancies.htm

DHHS and NIH are
Equal Opportunity Employers

MOLECULAR AND CELLULAR NEUROSCIENTIST
The M.I.N.D. Institute and an Academic Department
(to be identified at a later date)
UNIVERSITY OF CALIFORNIA, DAVIS, SCHOOL OF MEDICINE

The M.I.N.D. (Medical Investigation of Neurodevelopmental Disorders) Institute at the University of California, Davis, School of Medicine (and an academic department to be identified at a later date) are recruiting for one (1) full-time faculty position (Assistant/Associate/Full Professor In Residence) whose research interests are in the molecular and cellular mechanisms of early neurodevelopment. Candidate must possess a Ph.D. and/or M.D.degree and at least four years of productive postgraduate experience. Individual selected for the position will be expected to build a successful, independent research program and to achieve excellence in the teaching of basic sciences to medical and graduate students. In addition, this scientist should be committed to directing a substantial portion of their program to research in animal models of autism and related neurodevelopmental disorders. Investigators whose research incorporates stem cell technology in understanding neurodevelopmental disorders are encouraged to apply. The most important criteria in the consideration of applicants are: (1) a record of excellence, creativity, and initiative in research, which establishes a strong potential to build a vigorous and competitive research program; and (2) a demonstrated ability to communicate as a teacher. Candidates will have trained in modern methods of molecular and cellular neurosciences with substantial expertise using animal stem cell models of neuronal development. The Candidate will carry out basic research that will compliment existing collaborative programs at the M.I.N.D. Institute. Appointment in the appropriate UC Davis department will be made at the Assistant/Associate/Full Professor rank (In Residence series) based on experience. The position includes a highly competitive salary and laboratory start-up package.

Applicants pursuing other areas of biomedical research directly relevant to understanding autism and related disorders are also encouraged to apply. Candidates should send (1) curriculum vitae; (2) brief statement of research interests and plans; (3) names and contact information for at least five references to: **David G. Amaral, Ph.D., Director of Research, c/o M.I.N.D. Institute, 2825 50th Street, Sacramento, CA 95817**. This position will be "Open Until Filled". For full consideration, applications should be received by **August 1, 2005**.

The University of California is an Equal Opportunity/Affirmative Action Employer.

MICROBIOLOGIST
GS-12, Salary Range of $61,000 to $80,260

Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Viral Diseases, Viral & Rickettsial Zoonoses Branch is seeking a permanent, full-time **RESEARCH SCIENTIST** with demonstrated expertise in molecular virology to serve as a team member in the investigation of rabies. The laboratory is located at CDC's Roybal campus in Atlanta, Georgia. The incumbent will be responsible for multiple aspects of laboratory's research, including detection, characterization, and molecular epidemiology of lyssaviruses from outbreaks or sporadic cases, as well as training and liaison duties with countries in the former Soviet Union. Candidates should have a Ph.D. or equivalent degree in virology, microbiology, biology, chemistry, biochemistry, genetics, or a closely related field, with at least two years postdoctoral experience and linguistic skills in English and Russian. Salary is commensurate with experience. Excellent benefits and federal retirement plan.

For further information, contact: **Dr. Charles Rupprecht** at **telephone: 404-639-1050** or by **e-mail: cyr5@cdc.gov.** Additional information about the position and application procedure can be obtained via the CDC **website: http://www.cdc.gov** or at the Office of Personnel Management **website: http://www.usajobs.opm.gov.** Interested persons should apply under announcement number DE2-05-3132. The application period will open on May 3, 2005, and will run until May 23, 2005. *HHS/CDC is an Equal Opportunity Provider and Employer.*

RENAL PATHOLOGIST
Northwestern University

The Department of Pathology at the Northwestern University, Feinberg School of Medicine, Chicago, invites applications for a tenure-track or tenured faculty position (**ASSISTANT, ASSOCIATE,** or **FULL PROFESSOR**) in diagnostic renal pathology. Rank and remuneration for this full-time position will be determined by qualification and experience. The start date of this position is September 1, 2005. Candidates must have an M.D. or M.D./Ph.D., be board-certified/eligible (AP or AP/CP) and eligible for an unrestricted medical license in the state of Illinois. This position will share the renal biopsy service responsibilities with another pathologist. Candidates should have research interests in developmental biology or genetics or cancer biology. Candidates are expected to develop a strong, externally funded research program and participate in the teaching mission of the Department. Please submit your curriculum vitae and names of three references via e-mail to: **Janardan K. Reddy, M.D., Chair, c/o Nancy Starks** at **e-mail: nstarks@northwestern.edu** by June 30, 2005.

Northwestern University is an Equal Opportunity/Affirmative Action Employer. Women and minority candidates are encouraged to apply. Hiring is contingent upon eligibility to work in the United States.

POSTDOCTORAL FELLOW position available immediately to study the mechanisms of apoptosis and cancer biology/cancer molecular genetics in the Department of Pathology, Louisiana State University Health Sciences Center, New Orleans, Louisiana. Candidates with a Ph.D. degree or equivalent with experience in cancer biology and/or cancer molecular genetics are encouraged to apply. Please send curriculum vitae and contact information for three references to: **Dr. Daitoku Sakamuro, e-mail: dsakam@lsuhsc.edu.** *LSUHSC is an Affirmative Action/Equal Opportunity Employer.*

The Department of Nutrition and Food Science of Auburn University is seeking candidates for the position of **RESEARCH FELLOW.** The individual in this position will perform diabetes/obesity research to characterize novel protein in insulin action and insulin resistance. Review of applications begins after May 20, 2005. For full announcement, refer to requisition No. 21082 and apply online at: **website: http://www.auemployment.com.** *Auburn University is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

OXIS International, Inc.

DIRECTOR, RESEARCH & DEVELOPMENT

OXIS International, Inc., is a Portland company specializing in the development and manufacture of research assays focused on oxidative stress research.

This key position actively directs research and new product development and oversees the activities of the research department.

Experience in oxidative stress research and in vitro diagnostic assay development highly desirable. Ph.D. in chemistry or similar laboratory science and 10 years of laboratory experience at a managerial level are preferred. Send resume, cover letter, and salary history by **e-mail: resumes@oxis.com** or by **fax: 503-283-4058. Website: http://www.oxis.com**.

FACULTY POSITION
MOLECULAR ONCOLOGY and GENETICS
The George Washington University Medical Center

The Department of Biochemistry and Molecular Biology is accepting applications for a tenure-track **ASSISTANT** or **ASSOCIATE PROFESSOR**. The successful candidate will have an outstanding record of research accomplishment using state-of-the-art genetic and/or genomic technologies to decipher molecular pathways involved in cell survival/death, tumor vascular biology, or tumor invasion and metastasis. Through a generous endowment, the Department is expanding in the area of biochemical genetics, including significant capital investments in microarray proteomics, and cluster computing core facilities. The successful candidate will become a member of both The George Washington University (GWU) Cancer Institute and the McCormick Genomics Center. The GWU Medical Center is a partner with the nearby Children's National Medical Center, The Institute for Genomic Research (TIGR), and the NIH research campus. The successful candidate will have an extramurally funded research program and will provide education and training to students and fellows in medical and graduate programs. Competitive salary and startup funds are available for this position. Faculty rank and salary will be commensurate with prior experience. Applicants with a Ph.D. and/or M.D. degree or equivalent degree and substantial postdoctoral experience should submit curriculum vitae, a detailed statement of research accomplishments and future research plans, funding history, a description of teaching experience, and contact information for three references to: **Biochemistry Search, The George Washington University Medical Center, Ross Hall 530, 2300 Eye Street, N.W., Washington, DC 20037**. Review of applications will begin June 15, 2005, and will continue until the position is filled. *The George Washington University is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL RESEARCH ASSOCIATE: NEUROPROTECTION

The Center for Neuroscience and Regeneration Research at Yale University has a position open for a Research Associate or Postdoctoral Fellow with Ph.D. and/or M.D. degree and with experience in neuroscience to join multidisciplinary research group studying neuroprotection in multiple sclerosis and spinal cord injury. Experience with animal models of demyelination, especially experimental allergic encephalomyelitis, is desirable, and experience with behavioral, electrophysiological, and/or quantitative morphological assessment of central nervous system injury is essential. Superb opportunity to work with highly interactive, collaborative team. Please send curriculum vitae, three letters of reference, and statement of interest to: **Stephen G. Waxman, M.D., Ph.D., Chair, Department of Neurology, LCI 708, Yale University School of Medicine, P.O. Box 208018, New Haven, CT 06520-8018.** *Qualified women and members of underrepresented minority groups are encouraged to apply. Affirmative Action and Equal Opportunity Employer.*

POSITIONS OPEN

FACULTY POSITIONS
MOLECULAR VIROLOGY

The Department of Microbiology at University of Texas Southwestern Medical Center (**website: http://www.utsouthwestern.edu/microbiology**) is seeking new faculty in molecular virology at the **ASSISTANT PROFESSOR** (tenure track) level. Faculty will be expected to develop front-rank, competitive, independent research programs that focus on one or more aspects of the viral life cycle (host-pathogen interactions, viral pathogenesis, disruption of viral replication, command of host cell processes, viral immunology, etc.) that will complement existing strengths in hepatitis C virus, West Nile virus, HIV/SIV, and viral oncogenesis. Research on any virus of medical relevance or that is a potential bio-threat is of interest. The candidate also is expected to contribute to the teaching of medical and graduate students. Attractive startup packages, including a competitive salary and new laboratory space, are available to conduct research in an expanding, dynamic environment. For exceptional candidates, an Endowed Scholars Program offers startup funds of $1,000,000 over four years. Candidates should have a Ph.D. and/or M.D. degree with at least two years of postdoctoral experience and an exceptional publication record. Candidates please forward curriculum vitae, three letters of recommendation, two or three representative publications, and a brief summary of future research to: **Dr. Michael V. Norgard, Chair, Department of Microbiology, University of Texas Southwestern Medical Center, 6000 Harry Hines Boulevard, Dallas, TX 75390-9048. Fax: 214-648-5905**. *U.T. Southwestern is an Equal Opportunity University.*

RESEARCH ASSOCIATE
PHARMACOGENETICS

A junior rank Research Associate position is available in the Department of Medicine at the University of Chicago to work on the pharmacogenetics of anticancer agents and gene mapping and regulation. Candidates must have a Ph.D. and three years of related research experience or related field experience. Experience in pharmacogenetics of epidermal growth factor receptor–targeting anticancer drugs, single nucleotide polymorphism discovery, genetics of UGT2B7 and UGT1A1-UGT1A9, polymorphism, human genetics, genotyping, genetic mapping and regulation is required. The candidate will be part of a multi-institutional research group (**website: http://www.pharmacogenetics.org**) which involves frequent interactions with a broad range of scientists with expertise in human genetics, bioinformatics, clinical pharmacology, and molecular pharmacology. The Department of Medicine at the University of Chicago provides a stimulating and interactive environment with several laboratories interested in diverse aspects of human variation, statistical and computational genetics, cancer genetics, and clinical pharmacology. Applicants should send curriculum vitae, a one-page letter describing their prior research experience, and current interests and goals and they should provide names and contact information of three references to: **Michelle Scheuer, e-mail: mscheuer@medicine.bsd.uchicago.edu.** *The University of Chicago is an Affirmative Action/Equal Opportunity Employer.*

ASSISTANT/ASSOCIATE PROFESSOR
PHARMACEUTICAL CHEMISTRY
North Dakota State University, Department of Pharmaceutical Sciences, Fargo

Tenure-track position starting on or after August 15, 2005. The successful candidate will be expected to establish an externally funded, independent research program, teach and mentor graduate students, and participate in courses offered in the Pharm.D. curriculum. A highly competitive salary and a startup package commensurate with qualifications and experience are available. More information at **website: http://pharmsci.ndsu.nodak.edu/jobs/index.html.**

European Science Foundation (ESF)

Call for EUROCORES themes

ESF is looking for new ideas for collaborative research at the European level

The European Science Foundation (ESF) is an association of 78 member organisations in 30 European countries devoted to the coordination, implementation, networking and science policy development in the basic sciences *(www.esf.org)*. The ESF wishes to contribute to the European Research Area with its EUROCORES Scheme. It is inviting well developed proposals for new EUROCORES Programmes *(EUROCORES themes)*.

The EUROCORES Scheme

The aim of the European Science Foundation Collaborative Research (EUROCORES) Scheme is to enable researchers in different European countries to develop collaboration and scientific synergy in areas where European scale and scope are required for leading-edge research. This should create the critical mass necessary for scientific excellence. The scheme provides a flexible framework which allows national basic research funding organisations to join forces to support top class European research *in and across all scientific areas.*

Eligibility criteria

Proposing groups must include scientists and/or representatives from national funding organisations from at least 4 different countries within ESF membership.

Criteria for the selection of EUROCORES themes

- Scientific quality, novelty and feasibility of the EUROCORES theme proposal
- Requirement for European collaboration
- Relationship to other ongoing/planned research initiatives in the field (national, European, international)
- Qualification of the proposers
- Appropriateness of funding requested

How to submit a EUROCORES theme proposal

EUROCORES theme proposals must be received by **15 June 2005 (midnight)** by e-mail in one single pdf attachment to *eurocores@esf.org*. The title of the EUROCORES theme should appear in the subject line.

The full Call with detailed information and proposal guidelines can be found at: *www.esf.org/eurocores* or contact:

EUROCORES Scheme – *eurocores@esf.org*

The EUROCORES Scheme is supported by the EC Sixth Framework Programme under Contract no. ERAS-CT-2003-980409.

The U.S. Environmental Protection Agency is recruiting for a **Microbiologist, GS-12/13/14** for the National Decontamination Team, Cincinnati, Ohio. Position serves as Agency expert in the area of microbiology to provide unique response capabilities for determining decontamination strategies at environmental emergencies and terrorist incidents involving traditional and weaponized biological agents.

Salary Range: $62,918 to $114,941 per year.

Announcement is open to all U.S. citizens from **April 15, 2005** to **June 15, 2005**. Applicants will apply for this vacancy online at **http://www.epa.gov/ezhire** website BEFORE MIDNIGHT Eastern Daylight Savings Time on the closing date of the announcement. You may also access the vacancy announcement at **http://www.usajobs.opm.gov**.

The U.S. Environmental Protection Agency is an Equal Opportunity Employer.

Professor and Head
Department of Cell Biology and Anatomy
Louisiana State University Health Sciences Center
School of Medicine
New Orleans

The Louisiana State University Health Sciences Center School of Medicine in New Orleans invites applications and nominations for Professor and Head of the Department of Cell Biology and Anatomy. The School is currently undergoing a period of extraordinary expansion, with unprecedented investments by the state of Louisiana in the further development of biomedical sciences in collaborations between the School of Medicine and internal and external agencies. The position presents the opportunity to create a new level of interdisciplinary research and collaboration in a department with complementary and diverse areas of expertise that include systems and developmental neurobiology, embryology, and developmental biology. The Department currently lists 22 full time faculty, 10 faculty with adjunct appointments, and 15 students in Ph.D. or M.D./Ph.D. programs. Many of the full-time faculty hold joint appointments in the Centers of Excellence for Neuroscience, Molecular and Human Genetics, Oral and Craniofacial Biology, Cancer, and Alcohol and Drug Abuse. The Department offers a graduate program in Development, Cell, and Neurobiology as well as Clinical Anatomy and participates in Interdisciplinary programs for Neuroscience and Biological Systems. The successful candidate will have a Ph.D. and/or M.D., an internationally recognized research program with a history of strong funding, demonstrable leadership ability, a proven commitment to education and research, and the ability to provide vision for the Department that builds on its historic strengths. Achievements in teaching, multi-disciplinary collaborative research, mentorship, and administration that promote an inclusive environment are essential. Additional information regarding the Department and Health Sciences Center can be obtained at **http://www.medschool.lsuhsc.edu/cell_biology/**.

Candidates should provide a *curriculum vitae* including a full list of publications, past and current research support, and a brief statement of educational, research, service, and administrative interests. These materials should be forwarded electronically to: **Dr. Arthur L. Haas, Chair, Cell Biology and Anatomy Search Committee, LSUHSC School of Medicine, Department of Biochemistry, 1901 Perdido Street, New Orleans, LA 70112; CellBiology-Anatomy@lsuhsc.edu.**

Review of applications will commence **23 May 2005** and will continue until the position is filled.

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HARC

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POSTDOCTORAL FELLOW
Environmental Toxicogenomics

A Postdoctoral Fellow position is immediately available for leading-edge research evaluating the safety of nanomaterials using a combined toxicogenomics/systems biology approach. The candidate should be a Ph.D. in biochemistry, molecular biology, or related field. Research experience in genetic or molecular toxicology and/or in mammalian cell and tissue cultures is a plus. The project is led by **Dr. Mary Jane Cunningham.** Houston Advanced Research Center (HARC) is a nonprofit, private research institute located in The Woodlands, Texas, just north of Houston (**website: http://www.thewoodlandstx.com**). We are focused on applying sustainability solutions to improve human life while protecting the environment for future generations. Send your curriculum vitae along with three references through the HARC **website: http://www.harc.edu/jobs**; click on the job title; follow directions for resume submission. No telephone calls, please.

Equal Opportunity Employer, Minorities/Females/Veterans/Persons with Disabilities.

RESEARCH POSITION, EXPERIMENTAL SOLID STATE PHYSICS

CEA-Grenoble seeks an experimentalist, Ph.D. in physics with postdoctoral experience, to develop electronic transport measurements on quantum coherent phenomena in low dimensional systems like semiconducting nanowires. Skills in advanced nanofabrication and very-low temperature instrumentation are welcome.

Applications with curriculum vitae, references, list of publications, and summary of research interests can be sent before June 15, 2005, to: **Dr. Louis Jansen, DRFMC, CEA-Grenoble, 17 rue des Martyrs, F-38054 Grenoble Cedex 9, France.**

For information, **e-mail: louis.jansen@cea.fr** or **e-mail: marc.sanquer@cea.fr.**

Visit **website: http://www-drfmc.cea.fr/.**

Western University of Health Sciences has an immediate opening for a **POSTDOCTORAL FELLOW** to work in the laboratory of **Professor N. A. Darmani** on vomiting circuits at the College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, Pomona, California, U.S.A. Must have a Ph.D. in neuropharmacology or neuroscience and significant publication in high performance liquid chromatography–electrochemical detection, microdialysis, stereotaxic surgery, enzyme-linked immunosorbent assay, and animal behaviors. Send curriculum vitae, a list of three references, and a description of previous experience to: **e-mail: cdabbs@employment@westernu.edu.** To view a complete job description, please go to **website: http://www.westernu.edu.**

Equal Opportunity Eemployer.

POSTDOCTORAL RESEARCH in molecular parasitology or immunology at Tulane. One skilled postdoctorate researcher to study mitogen activated protein kinase signaling and one for immunology in toxoplasma and plasmodium. Requires expertise in molecular biology or immunology plus tissue culture skill and mouse experience. Curriculum vitae, statement of interests, and three references to: **Michael Brumlik, e-mail: mbrumlik@tulane.edu.** *Tulane University is an Affirmative Action/Equal Opportunity Employer.*

SENIOR POSTDOCTORAL FELLOW/RESEARCH ASSISTANT PROFESSOR position is available in the translational neuroscience program for studies in Alzheimer's and Parkinson's diseases. Interested candidates should send curriculum vitae to: **Isabela Diaconescu, M.S., Program Manager, Department of Psychiatry, The Mount Sinai School of Medicine, New York City, New York; e-mail: isabela.diaconescu@mssm.edu.**

POSITIONS OPEN

School of Fisheries and Ocean Sciences
University of Alaska Fairbanks

Responsibilities: The University of Alaska Fairbanks (UAF) is seeking candidates for a senior position in the School of Fisheries and Ocean Sciences. This position combines executive duties as **DIRECTOR** of the West Coast and Polar Regions Undersea Research Center, one of six regional centers in the National Oceanic and Atmospheric Adminstration's Undersea Research Program (NURP), with faculty and administrative duties as **DIRECTOR** of the Global Undersea Research Unit, an academic research unit within the School. This is a regular, full-time, exempt, tenure-track position.

Qualifications: An earned doctorate in marine or fisheries science or a related field, an active research program in a benthic marine science or fisheries science, and experience with undersea vehicles and technology used by the WCPR Center. See the full announcement and application at **website: http://www.uaf.edu/uafhr/Emp_Opp.html.**

Salary: competitive. Closing date: open until filled. First review date: August 1, 2005.

For information, contact: **Dr. Jennifer Reynolds, telephone: 907-474-5871; e-mail: jreynolds@guru.uaf.edu.** *The University of Alaska Fairbanks is an Equal Employment Opportunity/Affirmative Action Employer and Educational Institution.*

CONSULTANT COMPUTATIONAL PROTEOMICS

The University of Minnesota Supercomputing Institute seeks to hire a Biological Computation User Support staff member. As a member of the Biological Computation User Support staff, the successful candidate will provide technical expertise in the area of proteomics and protein science. The successful candidate will be responsible for identifying and supporting high throughput mass spectrometry software that identifies proteins and maps them to genes and to their biological function; for developing microarray and massarray database models that can be integrated with various genomic, protein structural, biological function, and chemical databases; and for developing an integrated approach to proteomics that will help researchers in characterizing and using protein sequence data.

This is an exciting opportunity to participate in a successful program that provides technical support to a broad range of development efforts and applications in computational biology. For details and to apply, please see our employment section at **website: http://www.msi.umn.edu** or contact: **Ann Johns, e-mail: johns@dtc.umn.edu** or **telephone: 612-624-1556.** *The University of Minnesota is an Equal Opportunity Educator and Employer.*

SENIOR DIRECTOR
Marine Management Areas Science Program
Washington, D.C.

Four years significant funding initiates cutting-edge research on marine management area effectiveness and MMA design. With a distinguished advisory committee, Conservation International staff, and partners, Director implements research which will benefit MMA management generally, but will be focused in developing countries expanding their marine management regimes. Applicants with strong records of accomplishment in biological or social science research in marine protected areas, with significant management and fund-raising skills, are particularly desirable. Submit resumes to: **Dr. Tom Lacher, Conservation International, Suite 600, 1919 M Street, N.W., Washington, DC 20036. Website: http://portals.conservation.org/marine/.**

POSTDOCTORAL POSITION available in neurobiology of neurodegeneration, including Alzheimer's disease. Strong background desired in biochemistry, molecular biology, and neurophysiology to explore the molecular basis of these diseases. Submit curriculum vitae and three references to: **Carol Miller, M.D., University of Southern California School of Medicine, Department of Pathology, 2011 Zonal Avenue, Los Angeles, CA 90033. E-mail: carolmil@usc.edu.**

POSITIONS OPEN

Science @ CAREERS
www.sciencecareers.org

Additional job postings not featured in this issue can be viewed online at **website: http://www.sciencecareers.org.** New jobs are added daily!

Manage your job search more effectively by creating an account at **website: http://www.sciencecareers.org.** You can post your resume (open or confidentially) in our database and use it to apply to multiple jobs simultaneously. Track the jobs you have applied to in special tracking folders. Plus, you can create Job Alerts that will e-mail you notification of jobs that match your search criteria.

SENIOR FACULTY POSITION IN NEUROSCIENCE
University of Virginia

The Department of Neuroscience at the University of Virginia is recruiting a new Senior Faculty member.

Candidates should have a Ph.D. and/or M.D. with a background in cellular, molecular, and/or systems aspects of the neurosciences. The University of Virginia is a unique academic environment with a longstanding tradition of interdisciplinary collaboration and faculty collegiality. Institutional strengths include outstanding neuroscience faculty with well-funded, cutting edge research programs, and keen interests in developing functional collaborations. Outstanding contributions to these research endeavors come from Ph.D., M.D./Ph.D. (M.S.T.P.), and undergraduate programs in the neurosciences.

The Charlottesville and central Virginia area offers an outstanding community for both personal and professional activities. A strong sense of community exists in the region along with excellent educational systems for adults and children. Charlottesville is located in the foothills of the Blue Ridge Mountains, and is consistently listed among the top cities in national rankings of the most desirable places to live. It offers a thriving community of the arts, highlighted by regular literary, film, theatrical, and historical festivals.

Applicants should submit curriculum vitae, a summary of current research activities, and the names and telephone numbers of three references to:

Faculty Search Committee
Department of Neuroscience
University of Virginia Health System
P.O. Box 801392
Charlottesville, VA 22908

The University of Virginia is an Equal Opportunity/Affirmative Action Employer.

POSTDOCTORAL POSITION
Theoretical Division

Los Alamos National Laboratory is currently accepting applications for a Postdoctoral employee to work on the Theoretical Division's project "Rational Approaches to Vaccine Design." The ideal candidate will have a strong background in computational biology, including phylogenetic analysis and other sequence analysis approaches, and have good coding skills in C/Unix. Ph.D. in related field required. Candidates more than five years past their Ph.D. will not be considered for a postdoctoral position.

A good statistical background and/or machine learning background along with a proven ability to analyze biological data are a plus. Candidates not meeting the above description of required job skills, but having related skills and a strong interest in computational biology will also be considered. To apply: Please send a comprehensive cover letter, curriculum vitae, and three letters of recommendation to: **Los Alamos National Laboratory, P.O. Box 1663/Mailstop B213, Los Alamos, NM 87545, Attn: Alan Lapedes.** *Equal Employment Opportunity.*

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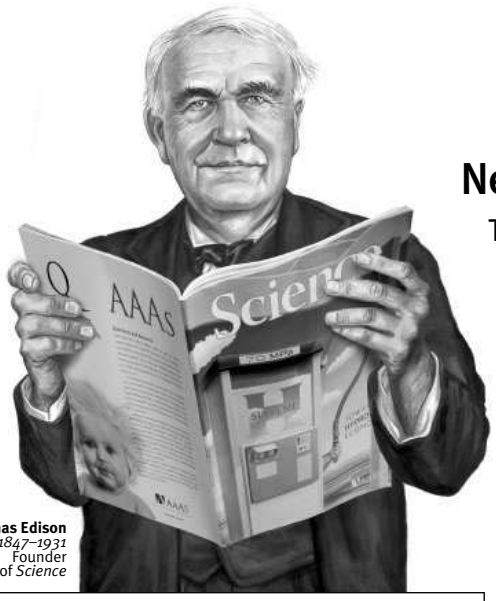

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